EFFECTS OF FRUIT TEMPERATURE, CALCIUM, CROWN AND SUGAR METABOLIZING ENZYMES ON THE OCCURRENCE OF PINEAPPLE FRUIT TRANSLUCENCY

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ABSTRACT

The role of preharvest fruit temperature, calcium, crown and sugar metabolizing enzymes in the occurrence of pineapple fruit translucency was studied. In Hawai'i, pineapple fruit translucency began to appear 4 to 2 weeks before commercial harvest. Pineapple fruit flesh became very susceptible to high temperature 6 to 4 weeks before harvest as flesh cell electrolyte leakage increased. High fruit temperature during the last stage of pineapple fruit development decreased titratable acidity and increased translucency.

The calcium concentration in pineapple fruit flesh declined with fruit development, possibly due to a decrease in the proportion of water imported via the xylem compared to the phloem. Mature fruit flesh tissue had a significantly reduced ability to bind divalent cations. Spraying calcium during pineapple fruit development decreased translucency occurrence at harvest.

Removing the crown either at an early or late stage of pineapple fruit development did not cause any significant effect on the fruit weight and translucency, suggesting that the crown did not play a significant role in pineapple fruit development and translucency occurrence. Defoliation conducted 4 or 3 weeks before harvest did not significantly reduce the pineapple fruit weight, but did significantly reduce the total soluble solids and fruit translucency, suggesting that the photoassimilate partitioning during the last stage of fruit development played an essential role in the occurrence of translucency.

Sucrose began to accumulate rapidly in pineapple fruit flesh 6 weeks before harvest, while the activities of three invertases and sucrose synthase were low. The activity of cell-

wall invertase (CWI) increased in the fruit flesh again 4 weeks before harvest, followed by the occurrence of translucency. There was a positive correlation between the CWI activity and translucency severity in pineapple fruit flesh, suggesting that the high CWI activity, that favored apoplastic phloem unloading, may be one of the causes inducing pineapple fruit translucency.

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LIST OF ABBREVIATIONS

AI	Acid invertase
BSA	Bovine serum albumin
CWI	Cell-wall invertase
DTT	DL-Dithiothreitol
E-64	trans-epoxysuccinyl-L-leucylamido-(4-guanidino) butane
EDTA	Ethylenediaminetetraacetic acid
HPLC	High performance liquid chromatography
MOPS	3-[N-Morpholino] propanesulfonic acid
NI	Neutral invertase
PMSF	Phenylmethylsulfonyl fluoride
PVPP	Polyvinyl-polypyrrolidone
SPS	Sucrose-phosphate synthase
SS	Sucrose synthase
TA	Titratable acidity
TSS	Total soluble solids
UDP	Uridine 5'-diphosphate
UDPG	Uridine 5'-diphosphoglucose

CHAPTER 1

INTRODUCTION

Pineapple (*Ananas comosus* L. Merr.) is one of the most economically important crops in tropical and subtropical areas and most (70%) is consumed fresh (Leal and Coppens d'Eeckenbrugge, 1996). Fruits for the fresh market are hand-harvested and handled with care to avoid mechanical injury (Nakasone and Paull, 1998) that could lead to fruit rot.

Translucency is a physiological disorder of the pineapple fruit flesh, in which the flesh shows water soaking and has low porosity. The intercellular free spaces in translucent fruit flesh are filled with liquid that reduces the light scattering ability of the tissue, making it translucent (Sideris and Krauss, 1933a). Translucent fruits are extremely fragile, making these fruits very prone to mechanical damage during harvest and postharvest handling (Py et al., 1987) and they are therefore difficult to ship fresh. In addition, translucent fruits are more susceptible to diseases (Gortner et al., 1963) and preharvest sunburn (Keetch, 1977).

The cause of translucent fruit is unknown. As with the similar condition of watercore of apple fruit (Malow and Loescher, 1984), translucency in pineapple is likely to be due to interactions of several factors. Translucency in Hawai'i has been associated with cultivar, high nitrogen, large vigorous plants, spring ripened fruit, treatment with fruit enlarging agents, irrigation rate, planting density, and environmental factors (Paull and Reyes, 1996). Paull and Reyes (1996) showed that both crown weight and fruit translucency at harvest were correlated to the monthly average air temperature three months before harvest, and the correlation between crown weight and translucency severity was significantly negative. The

crown may affect translucency occurrence by competing with the fruit for assimilates and shading the fruit to reduce fruit temperature during sunny days.

The objectives of this study were to examine the effects of crown and fruit temperature on the occurrence of pineapple fruit translucency, to determine the relationship between sugar metabolizing enzymes and translucency of fruit flesh, and to examine the effect of calcium application during fruit development on the occurrence of translucency at harvest.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Physiological effects of high temperature on membrane integrity and carbon partitioning in plants.

Temperature is one of the most important environmental factors that regulates plant growth and development (Fitter and Hay, 1987). High temperature can be a primary limiting factor in the distribution and adaptability of wild and cultivated plants (Ranney et al., 1995). Plants growing in semiarid environments routinely encounter high radiation, water deficit, and high day temperature during all or part of their life cycles (Burke, 1990). At the cellular level, heat stress results in metabolic disturbances, depletion of respiratory substrates, reduction of photosynthetic activity, denaturation of proteins, enzyme inactivation and damage to cellular structures (Nguyen et al., 1994).

It is widely accepted that membranes play a key role in the response of cellular functions to variable stress conditions (Hale and Orcutt, 1987). The heat-tolerance limits of various plants and microorganisms differ over a wide range, and considerable differences exist in the thermostability of individual cellular membranes and their adaptability to supraoptimal temperature (Santarius and Weis, 1988). High temperature can modify membrane composition and structure (Björkman et al., 1980). When a cellular membrane is injured by exposure to high temperature, cellular membrane permeability is increased, and electrolytes leak out of the cell (Björkman et al., 1980). An increase in solute leakage, indicating a loss of selective permeability is also the most obvious indication of membrane change found during

senescence (Ferguson, 1984). Changes in growth temperature are associated with changes in membrane lipid composition in plants (Quinn, 1988). Acclimation to high temperature was suggested to be associated with a greater degree of saturation of fatty acids in membrane lipids, tending to make the membrane less fluid (Raison et al., 1982; Quinn, 1988).

Carbon partitioning within sources and sinks and carbon transport between them has been reviewed in relation to temperature (Farrar, 1988). Temperature can affect sink metabolism simply by speeding or slowing individual reactions; by altering rates of diffusion, particularly where diffusion is a necessary and possibly rate-limiting part of active transport across membranes; and by more specific effects that include coarse control of enzyme complement (Farrar, 1988). Sink development may respond to accumulated temperature (Johnson and Thornley, 1985), and temperature-induced changes in the source, sink and phloem will inevitably have effects on assimilate partitioning between organs (Farrar and Williams, 1991). Higher temperature increases sugar accumulation in Satsuma mandarin fruit during development (Richardson et al., 1997). Warming an individual sink increases assimilate import into it (Farrar, 1988) and stimulates the sink's growth rate (Marcelis and Baan Hofman-Eijer, 1993). There is a positive correlation between the carbon import rate of a tomato fruit and the fruit temperature (Walker and Ho, 1977). However, increases in temperature above the optimum for growth impair sink metabolism and sugar accumulation, and further import is reduced (Farrar and Williams, 1991).

2.2 Physiological roles of calcium in plants.

Calcium has diverse physiological roles in plants. An important role is the

maintenance of membrane integrity (Leshem, 1992), and middle lamella and cell wall rigidity (Brett and Waldron, 1996). Calcium may constitute a structural element in membrane architecture, and at low concentrations functions as a stabilizer, while at high concentration it leads to a rigid structure (Leshem, 1992). Calcium also plays a key role in signal transduction in plants, acting as second messengers (Poovaiah and Reddy, 1993; Webb et al., 1996). Changes in cellular Ca^{+2}, acting through Ca^{+2}-modulated proteins and their targets, regulate a variety of cellular processes during plant growth and development (Bush, 1995).

Many physiological disorders of plants are related to the calcium content of the respective tissues or organs (Bangerth, 1979). A localized inadequacy of calcium can induce a variety of disorders of fruit and vegetables (Shear, 1975). Optimal calcium content and proper distribution in individual plant organs can prevent the occurrence of many physiological disorders caused by unfavorable external conditions (Poovaiah, 1993).

Calcium plays a significant role in controlling fruit softening and ripening (Poovaiah et al., 1988; Ferguson, 1984). Fruit maintaining relatively high calcium content in flesh tissue has a slower rate of ripening and softening (Ferguson, 1984). High calcium concentration may increase calcium ion binding to pectic polymers that reduces the rate of pectic solubilization (Ferguson, 1984), and decreases the secretion or activities of cell wall hydrolases (Huber, 1983).

It is generally accepted that the presence of calcium ions increases the cohesion of the cell walls (Demarty et al., 1984). Calcium ions are fixed in the cell wall by both electrostatic interactions with the carboxylic groups of the pectin, and coordination linkages with hydroxylic groups of diverse polysaccharides (Brett and Waldron, 1996). Concerted ionic

binding may occur cross-linking unsubstituted polygalacturonic acid via calcium ionic bridges to other negatively charged galacturonate residues (Brett and Waldron, 1996), forming the egg-box structure (Morris et al., 1982).

Calcium ions form electrostatic bridges either between individual neighboring anionic phospholipids, or between the latter and membranes, or with cytoskeletal elements (Leshem, 1992). Calcium ions also counteract stress associated phenomena in fruit, an effect attributed to membrane stabilizing action. Preharvest calcium sprays and postharvest pressurization of calcium have been used to increase fruit firmness and reduce decay or calcium-related disorders (Leshem, 1992).

The xylem is generally considered to be the major pathway of calcium translocation in plants (Clarkson, 1984). During the early fast growing stage, apple fruit has a high water demand to maintain turgor pressure needed for cell expansion (Wiersum, 1966). As the fruit continues to enlarge and start to accumulate assimilates, photoassimilates from the source leaves imported into the fruit increases, leading to a major shift in the route of water supply from the xylem to the phloem. Calcium in the phloem is relatively immobile (Himelrick and McDuffie, 1983). This greatly restricted calcium uptake via the phloem can not keep pace with fruit expansion and assimilate accumulation, and the calcium concentration within the fruit declines as it is progressively diluted (Wiersum, 1966; Ferguson and Watkins, 1989). During tomato fruit growth and development, the daily calcium accumulation rate also decreases with the decline in the proportion of water imported via the xylem (Ho et al., 1987).

Disorders caused by an inadequate calcium content in affected tissues cannot be corrected simply by supplying additional calcium to the soil (Shear, 1975), because there is

no guarantee that calcium supplied to the soil will be transported to the affected tissues. For example, blossom-end rot is a local deficiency of calcium in tomato fruit, frequently occurring even when the moisture and calcium content of the substrate are fully adequate in the root zone (Adams and Ho, 1993). Poor calcium uptake by the root and/or inadequate distribution of calcium to the fruit at a period of high calcium demand are more likely the causes (Adams and Ho, 1993). As a result, to correct disorders caused by calcium deficiency, the entire system of uptake, translocation and distribution must be considered and the necessary adjustments must be made to assure adequate calcium accumulation in affected tissues (Shear, 1975). Sprays of calcium solution may supply sufficient calcium to control disorders in fruits. However, the calcium from sprays must move into fruits through their surface, and only very limited quantities can be supplied in this way (Shear, 1975). Therefore, for each fruit species, the timing of spray and the calcium formulation must be considered in order to obtain an efficient result.

2.3 Overview of carbon partitioning in plants.

Carbon partitioning in the whole plant is dependent upon two processes, the rate of import into a sink and the proportional distribution of assimilates between competing sinks (Farrar, 1988). These processes are affected by the photosynthetic capacity of source leaves and the competitive ability and demand for assimilates by sink organs, the nature of the vascular linkages between sources and sinks, and the capacity for temporary storage by tissues (including the source) along the transport pathway (Wardlaw, 1992).

Phloem loading is an important step in the transport pathway of photoassimilates. Two main modes of phloem loading have been proposed, apoplastic and symplastic (van Bel, 1992a). There is a coincidence between the mode of phloem loading and the plasmodesmatal configuration of minor-vein (van Bel et al., 1992), that has been suggested to be correlated with the climate (van Bel, 1992a; van Bel and Gamalei, 1992). A paucity of plasmodesmata in a phloem-loading pathway supposedly reflects a symplastic constriction which provokes apoplastic transfer (van Bel, 1992a). The uneven distribution of minor-vein types between various terrestrial ecosystems indicates that apoplastic loading predominates in cold and dry climate zones, leading to the suggestion that drought and temperature stress have led to the transformation of the ancient symplastic mode into the more advanced apoplastic mode of loading. van Bel et al. (1994) have developed a three-step screening procedure to identify the mode of phloem loading in intact leaves. They found that phloem loading is associated with the type of companion cell, and suggested that species with transfer cells as companion cells, specialized for uptake of solute from the apoplast, occur via the apoplast and species with intermediary cells as companion cells, specialized for symplastic phloem loading, occur via symplast. Taken together, apoplastic transport is responsible for phloem loading in species in which sucrose alone is exported, and symplastic phloem loading may predominate in species in which a combination of sucrose and any other carbohydrate is translocated (Flora and Madore, 1996). A major difference between symplastic and apoplastic species might be the scantiness of raffmose (or other oligosaccharides) carriers in the mesophyll plasma membrane of species with the apoplastic mode of phloem loading (van Bel et al., 1996).

The long-distance phloem transport pathway between source and sink is through the sieve elements of the phloem. According to the Münch hypothesis, the osmotic gradient between the sieve element/companion cell (SE/CC) complex in source and sink is the driving force of phloem translocation (van Bel, 1992b). However, other solutes in the sieve element and the parallel gradient of water potential in the xylem might also play a role in the long-distance phloem transport. There is substantial passive leakage of photoassimilate from the sieve tubes along the path (van Bel, 1992b), and to maintain a high osmotic gradient, the SE/CC complex needs a highly active, energy dependent uptake system to balance the continuous efflux of solutes (van Bel, 1992b). The proton gradient required for the sucrose-proton cotransport is produced by a H^+-ATPase located in the plasma membrane of companion cells (Bouche-Pillon et al., 1994). Incoming sucrose can be cleaved by either sucrose synthase (SS) or cytosolic neutral invertase (NI). SS has been demonstrated to be directly involved in sucrose cleaving and metabolism during glycolysis within the phloem complex of *Ricinus communis* L. seedling (Geigenberger et al., 1993). Since sucrose is the main transport sugar in the phloem, the role of high activities of SS and NI is probably to produce hexoses for the production of ATP to support the sucrose-proton cotransport.

As with phloem loading, two modes of phloem unloading have been proposed, apoplastic and symplastic. Convincing evidence for apoplastic unloading exists in the transport of photoassimilates from the maternal tissues to developing seeds (Patrick, 1990). Symplastic unloading is prevalent where the photoassimilate is rapidly utilized for growth (utilization sinks) or polymer formation (storage sinks) (Patrick, 1990). Symplastic unloading is also conceivable in sink tissues where osmotically active sugar is stored at high

concentration in the vacuole (Niland and Schmitz, 1995), whereby a concentration gradient between the sieve element and the sink cell is maintained.

Sugar metabolizing enzymes are important in sugar metabolism and compartmentation in sink organs (Hawker, 1991). There is a differential temporal and spatial expression of SS during tomato fruit development (Wang et al., 1994). Differential expression of SS genes can be modulated by sugar levels in sink cells, providing a potential mechanism for altering the pattern of enzyme distribution and also for adjusting the sucrose metabolism capacity of the importing cells (Koch et al., 1992). The higher levels of SS mRNA and protein in tomato fruit are correlated with the rapid growth stage of fruit development and the vascular tissues (Wang et al., 1994). In addition, a significantly greater activity of SS in vascular tissue was also found in grapefruit, indicating that the role of this enzyme in translocation may include a vascular function (Tomlinson et al., 1991). A clear companion cell specificity is evident in the phloem unloading zone of citrus fruit, where high activity of SS exists in vascular bundles during periods of rapid sugar import (Nolte and Koch, 1993). Moreover, the companion-cell specificity of SS were also found in mature leaves of citrus and maize (Nolte and Koch, 1993), implying that this may be a widespread association and enhance its potential importance to the physiology of vascular bundles and phloem transport.

There is increasing evidence that invertases (soluble and cell-wall bound) may play an important role in carbon partitioning in plants. Deficiency in invertase activities in the maize mutant *miniature* is associated with aberrant pedicel and endosperm development (Miller and Chourey, 1992), indicating that invertase activities can directly affect the metabolic and developmental stability of the maternal cells. As with the SS genes, invertase genes are also

sugar modulated (Xu et al., 1996). Glucose and the nonmetabolizable glucose analog 6-deoxyglucose induce cell-wall invertase (CWI) gene expression of *Chenopodium rubrum* (Roitsch et al., 1995). In addition, both enzyme activity and mRNA level of CWI show a sink-tissue-specific distribution, suggesting that CWI not only has an important function in phloem unloading and carbon partitioning between source and sink, but may also have a role in the establishment of metabolic sinks (Roitsch et al., 1995).

In carrot, SS regulates sucrose utilization in developing tap roots, whereas vacuole invertase controls sucrose storage and sugar composition (Sturm et al., 1995). In addition, similar regulatory patterns occurring in these two paths of sucrose metabolism, catalyzed by SS and invertases, have been found in maize (Xu et al., 1996), indicating a potential to regulate carbon allocation. Pradel et al. (1996) found that symplastic phloem unloading occurs in *Agrobacterium tumefaciens*-induced plant tumors, and CWI activity is about tenfold higher in the same tumor tissue than in the adjacent host stem tissue, suggesting that phloem unloading may be independent of CWI activity. To confirm the role of CWI in phloem unloading, more detailed study is necessary.

Since the unloading pathways are complex in various sinks, it is difficult to distinguish postphloem, nonvascular transport experimentally from phloem unloading. Postphloem transport can be apoplastic or symplastic. The unusual anatomical feature of citrus juice sac tissues provides an opportunity to examine nonvascular transfer alone over a relatively long distance (Koch and Avigne, 1990). In citrus fruit, postphloem transport occurs against an ascending sucrose concentration gradient in young fruit, whereas a descending gradient develops later in maturation (Koch and Avigne, 1990). The cellular pathway of postphloem

sugar transport might also change during fruit development. In the tomato outer fruit pericarp, the postphloem transport shifts from the symplast during starch accumulation (13-14 days after anthesis) to apoplast for rapid hexose accumulation (23-25 days after anthesis) (Ruan and Patrick, 1995). In addition, an energy-coupled plasma-membrane hexose carrier is expressed specifically in storage parenchyma cells at the latter stage of tomato fruit development (Ruan and Patrick, 1995), indicating an apoplastic pathway.

A competitive sink must have a fine vascular linkage to the source and maintain a strong osmotic and pressure gradient between them (Ho, 1988). Active assimilate unloading into a sink will be enhanced by an extensive phloem unloading area, efficient membrane transfer and effective physical and/or chemical isolation of storage and growth products. It has been proposed that the activities of sink metabolism and compartmentation are the ultimate determinants of the turgor potential at the sink end of the phloem that drives mass flow of imported assimilate (Minchin and Thrope, 1987). The high sink strength of developing seeds may be due to the sink end of the phloem pathway being 'bathed' in an apoplastic solution having a high concentration of osmotically active solutes (Wolswinkel, 1990). Therefore, turgor-dependent unloading of assimilates from developing legume seed coats (Patrick, 1993) provides a mechanism to link sink demand for assimilates with the phloem translocation. Because of the great complexity of the unloading pathway in various sinks and the difficulty in determining the exact sites of carbon unloading, understanding the mechanisms and controls of unloading pathway are far from complete (Patrick, 1997). In addition, elucidation of the cellular pathway of phloem unloading in most sinks examined thus

far lacks the quantitative evaluation (Patrick, 1997), required before definitive conclusions can be drawn.

In the source-transport-sink system, phloem unloading in sink organs can be regulated by the sour e activity. On the other hand, the phloem loading in source leaves can be also regulated by the sink strength (Wardlaw, 1990). Both source and sink interact through the transport pathway to regulate the balance between supply and demand. Expression of a yeast-derived invertase in the cell wall of tobacco and *Arabidopsis* led to a decrease in sucrose export, accumulation of carbohydrate and inhibition of photosynthesis (von Schaewen et al., 1990), suggesting that photosynthesis in the source leaf is sink-limited. Upon continuous removal of flower buds, the sink-limited soybean plants showed a large decrease in the rate of photosynthetic CO_2 fixation with the inactivation of ribulose-1, 5-bisphosphate carboxylase (RuBPase) (Sawada et al., 1995). Photosynthesis is also feedback regulated by the accumulation of carbohydrates in source leaves (Goldschmidt and Huber, 1992), through the control of photosynthetic gene expression. Doubling the CO_2 concentration enhances the activities of carbohydrate metabolizing enzymes, carbohydrate production, photoassimilate transport, and sink strength (Wang and Nobel, 1996). In addition, it has been demonstrated that the sink capacity (i.e., the potential of the sink to accommodate assimilates) and source strength (i.e., the ability of the leaf canopy to produce assimilates) are highly interdependent (Wang et al., 1996). As a result, balance among photosynthesis, translocation, and sink metabolism is a significant factor in crop production.

2.4 Overview of pineapple fruit growth and development.

Pineapple is a quantitative short-day plant (Gowing, 1961; Friend and Lydon, 1979). Cool temperatures promote flowering of pineapple (Py et al., 1987), particularly cool night temperature (van Overbeek and Cruzado, 1948; Bartholomew and Malézieux, 1994; Min and Bartholomew, 1997). Flower induction (forcing) treatments are also more effective at cooler temperature (Bartholomew and Malézieux, 1994) and can fail at high temperature (Turnbull et al., 1993), especially when the high temperature occurs at night (Bartholomew and Malézieux, 1994). Furthermore, when forcing at high temperature is successful, fruit weight and eye numbers are significantly reduced (Min and Bartholomew, 1997). High night temperatures also cause a significant increase in crown weight, with the total weight of fruit and crown being unaffected (Min and Bartholomew, 1997). As a result, the effect of night temperature on dry matter production of total fruit and crown is not significant.

The crown is a short, shoot-like growth, bearing leaves which develop on the top of the fruit. It is a continuation of the original main axis meristem of the plant, after the formation of the inflorescence producing only floral bracts for a short time, it then reverts to leaf production giving rise to the crown (Collins, 1960). The crown and fruit are possible competitors for photoassimilates, nutrients, water, etc. However, applying chlorflurenol to reduce crown size did not affect the fruit size (Keetch and Dalldorf, 1980; Dalldorf, 1981). In contrast, fruit size may affect crown size since larger fruits generally have smaller crowns and vice versa, suggesting that fruit is a stronger sink than the crown. This is consistent with other observations that, in general, fruits are stronger sinks than the vegetative organs for assimilates (Ho, 1988).

The growth curve of crowns is sigmoid and the greatest growth occurs 3 to 1 month before harvest (Sideris and Krauss, 1938; Py et al., 1987). Higher temperature during this period may enhance crown growth. In Hawai'i, fruits harvested from October to December have higher crown weights than fruits harvested in other months (Paull and Reyes, 1996). This is due possibly to higher temperature and greater supply of photoassimilates during the fastest growth stage, i.e., the summer. Reduced solar radiation and lower air and soil temperatures from November to April in Hawai'i result in slower vegetative growth rates (Bartholomew and Kadzimin, 1977). Nightingale (1942) also reported that low soil temperature (20°C or lower) resulted in limited nitrate absorption by the roots. Those results imply that fruit and crown growth rates might be slower in the winter. The relative rates of leaf elongation and relative total dry matter production of 'Smooth Cayenne' pineapple at different thermoperiods is 30/26°C (average 28°C) > 26/22°C (average 24°C) > 22/18°C (average 20°C) (Bartholomew and Kadzimin, 1977). However, the developmental rate also decreases if the day temperature is above the optimum (Malézieux et al., 1994). Forcing at cool night temperature produces fruits with higher fruitlet numbers than forcing at warm night temperature (Min and Bartholomew, 1997) that may increase fruit growth, and reduce crown growth. Therefore, fruits harvested in spring have significantly smaller crowns (Paull and Reyes, 1996), due possibly in part to cool night temperature during the forcing treatment, that produces more fruitlets.

Pineapple is the only example of a widely cultivated commercial crop with crassulacean acid metabolism (Py et al., 1987). Carbon dioxide assimilation by the pineapple plant mostly occurs during the dark period (Sale and Neales, 1980), and high night

temperature reduces the total CO_2 assimilation (Neales, 1973; Neales et al., 1980). Starch or other carbohydrate reserves are not stored in quantity in unripe pineapple fruits. Shortly before ripening there is a rapid influx of sugar from the vegetative organs to the fruit, the rate apparently being controlled in large part by air temperature (Nightingale, 1942). When the average temperature during the week prior to picking is low (20°C), plants high in starch reserves (in that they produce fruit relatively high in sugars) have a decided advantage over plants comparatively low in carbohydrates (Nightingale, 1942). However, this advantage is negligible when the average temperature is higher (20.6°C-23.9°C) (Nightingale, 1942). When the average fruit temperature during the week prior to picking is high because of a high light level or a high degree of exposure to sunlight, or both, the fruit is low in acidity, conversely, low fruit temperature makes for higher acidity (Nightingale, 1942).

2.5 Development of pineapple fruit translucency.

Pineapple fruit translucency occurs before harvest and generally the basal flesh is the first to show symptoms; in severe cases the whole fruit is affected (Paull and Reyes, 1996). Translucency, as opposed to opaqueness, is a property of the fruit flesh tissue that lacks the presence of small air bubbles in the intercellular spaces of the tissue (Sideris and Krauss, 1933a). Since the air spaces are filled with liquid, translucent fruit has a higher specific gravity than opaque fruit (Sideris and Krauss, 1933a). The specific gravity of pineapple fruit increases with growth, with the specific gravity of mature fruit being higher than immature fruit. This is consistent with the occurrence of translucency in nearly mature fruit. The specific gravity can be employed as a nondestructive detection method of fruit translucency.

Translucent fruit is usually sweeter than opaque fruit (Sideris and Krauss, 1934) and has lower acidity while total soluble solids (TSS) remains about the same or even less (Gortner et al., 1963). Soler (1994) also reported that the TSS of translucent fruit is not significantly different from that of normal fruit, but the acidity of translucent fruit is significantly lower than that of normal fruit. Highly translucent fruit has flat and over-ripe off-flavors, and significantly lower edible quality (Bowden, 1967). Moreover, Bowden (1969) reported that optimum palatability of 'Smooth Cayenne' pineapple occurred when the fruit was semi-translucent (52%-54%).

Translucency can occur when the fruit shell is still green (Py et al., 1987). The green-shell translucency may be due to lesions in the cell walls caused by high temperatures and/or too abundant supplies of sap brought about by a sudden improvement in the water supply during a period of intense photosynthetic activity (Py et al., 1987). The physicochemical basis of green-shell translucent fruit is different from yellow-shell translucent fruit. In green ripe fruit, the development of flesh tissue translucency is independent of the reactions influencing the pigmentation of the shell tissues. This might indicate that the flesh tissue is maturing ahead of the shell tissue (Sideris et al., 1935), or both the development of flesh tissue and shell are independent of each other.

Gortner et al. (1963) summarized some factors that increase green-shell translucency including high nitrogen fertilization, heavy rainfall or irrigation, especially after a dry period; winter and spring-ripening fruit; and treating fruit with enlarging hormones. Green-shell translucency is found more frequently in large fruits and during high temperature periods (Green, 1963). Soler (1993) studied how the translucency of green-ripening differed at a

biochemical level. He found that translucency was characterized by an increase in catalase, and α- and β-galactosidase activities, that might be related to translucency by the modification of membrane galactolipids and permeability changes. Soler (1994) also reported that the conductance of translucent tissues was higher than that of normal tissues. This implies that the membrane permeability of translucent fruit cells is higher than that of normal fruit cells.

The thermal disruption of membrane integrity is a primary step in high temperature injury (Steponkus, 1981), and electrolyte leakage can be used as an indicator of high-temperature injury (Chen et al., 1982; Inaba and Crandall, 1988). High temperature can reduce cellular membrane integrity that could lead to leakage (Steponkus, 1981). Fruit tissues are leakier at high temperatures (Burg et al., 1964), leading to the flooding of intercellular spaces. Since green-shell translucency is found more frequently during high temperature periods (Green, 1963), therefore, high-temperature injury could be one of the causes.

Opaque fruits are smaller and have smaller eyes, and the plants on the inside rows of 4-row beds, accordingly shaded by their neighbors, tend to produce more opaque fruits (Linford, 1933). The fruits in the inside rows might have a lower fruit temperature that reduces the occurrence of translucency.

Generally, translucency occurs more frequently in large fruit. Translucency increases with increasing fruit weight (Bowden, 1969). The addition of nitrogen is also associated with an increase of fruit weight and translucency (Magistad and Linford, 1934b). Potash alone has very little effect on translucency, but when extra nitrogen is applied, the gains in translucency from potash are greater and more consistent (Magistad and Linford, 1934a). Increased fertilization, leads to an increase in fruit size and translucency, with shorter and narrower

crowns, agreeing with the common observation that larger fruits have smaller crowns (Linford, 1934b). There is a progressive increase in mean translucency rank with a decreasing number of plants per acre (Linford and Mehrlich, 1934), implying that less competition for nutrition and water, and less shading from neighbor plants increases translucency. Planting materials with higher initial weights, produce larger plants and fruits of corresponding high weights and eye numbers, and ripen earlier (Linford et al., 1934; Sideris and Krauss, 1935), and also produce more translucent fruits (Linford et al., 1934). Iron deficiency is frequent during pineapple production in Hawai'i and the application of iron is a necessary plantation practice that can increase fruit weight and eye number, but also increases translucency (Linford, 1934a). Irrigation in a low rainfall area increases fruit weight and percentage of translucent fruit, but reduces TSS and crown weight (Krauss, 1944).

High sugar content is generally associated with the summer months and high acid content with the winter months (Sideris et al., 1936). The acidity of winter harvested fruit increases regardless of the degree of translucency and the reverse is true for the summer harvested fruit (Sideris et al., 1936). Therefore, the months when fruits have the highest degree of translucency are not necessarily those with the lowest acidity. However, translucent fruits normally contain smaller quantities of citric acid than opaque ones in the same season (Sideris and Krauss, 1933a), but sugar content in both types of fruit does not significantly differ (Sideris and Krauss, 1933b).

Sideris and Krauss (1936) reported that defoliation led to fruit with lower weight and sugar content, and a higher amount of acidity. Nevertheless, the effects of defoliation on fruit translucency were very variable and no satisfactory conclusions were drawn. One reason for

this variability was possibly because that fruits in this trial were not harvested at the same date. The differences of harvest dates in different treatments were from one to three weeks.

Generally, translucency is associated with less flavor traditionally measured in terms of sugar, acid, and ester content. Translucent fruits are susceptible to pink disease, bacterial and yeast fermentation (Gortner et al., 1963). The incidence of peduncle leakage is correlated with translucency severity (Paull and Reyes, 1996). This leakage keeps the broken peduncle end wet and may lead to an unsightly dark bluish grey *Penicillium* growth postharvest (Paull and Reyes, 1996). Genetically, 'Smooth Cayenne' is susceptible to translucency if cultural and environmental factors are favorable (Gortner et al., 1963).

To date, the cause or causes of pineapple fruit translucency are unknown. As with the similar condition in watercore of apple fruit (Malow and Loescher, 1984), translucency in pineapple fruit is likely to be due to interaction of several factors including heredity, environment, source-to-sink ratio, fruit maturation and ripening.

CHAPTER 3

DEVELOPMENT OF HYPOTHESES

If there is a cause-effect relationship between crown size and pineapple fruit translucency, then artificial crown removal should increase fruit translucency occurrence since there is a highly negatively significant correlation between crown weight and translucency severity.

If the effect of crown size is due to shading fruit to reduce fruit temperature during sunny days, then artificial shading of fruit should have the same effect.

If high fruit temperature is a significant cause of translucency, then increasing fruit temperature during development should increase the occurrence of translucency and reduce fruit acidity at harvest as happens with translucent fruit in field.

If there is a cause-effect relationship between the monthly average air temperature at 3 to 1 month before harvest and fruit translucency at harvest, and the effect is due to the difference in fruit temperature, then modifying fruit temperature at 3 to 1 month before harvest should affect the occurrence of fruit translucency at harvest.

Calcium contributes to cell wall integrity, stabilization of cell membranes, and delays maturation and fruit ripening. If cell wall and membrane integrity is an important factor related to fruit translucency, then spraying calcium during fruit development should reduce the occurrence of fruit translucency at harvest.

Besides membrane leakage, sugar unloading into the apoplastic free spaces of fruit flesh might also cause the occurrence of fruit translucency, as sugar unloading into the free

spaces could increase apoplastic osmotic pressure; that in turn, causes the movement of water from the vascular tissues to the free spaces, leading to the occurrence of fruit translucency. Cleaving sucrose into glucose and fructose with cell-wall invertase (CWI), can stimulate the rate of sucrose unloading by increasing the sucrose gradient between the phloem and apoplast. In addition, when sucrose is broken down into reducing sugars, that can double the osmotic pressure contributed originally by sucrose. Therefore, high activity of CWI should favor the occurrence of fruit translucency. If the CWI activity is a factor related to translucency, then the activity of CWI should be high at the latter stage of fruit development, because the translucency of flesh is generally seen when fruit is nearly mature. In addition, the CWI activity in translucent fruit should be higher than that in the opaque fruit.

If high photoassimilate unloading into the fruit tissue can increase the occurrence of translucency, then leaf removal at the latter stage of fruit development should reduce the occurrence of fruit translucency.

In general, the basal fruit flesh is the first to show translucency. If the CWI activity is positively correlated to translucency, then the activity of CWI in the basal flesh should be higher than that in the top flesh.

Fruitlets generally show the translucent symptom earlier than interfruitlet tissues. If sugar unloading and CWI activity are important in translucency occurrence, then the sugar content and CWI activity of fruitlets should be higher than the interfruitlet tissues, particularly at the latter stage of fruit development.

The following hypotheses were tested in this study:

1. High fruit temperature at 3 to 1 month before harvest increases the thermotolerance and reduces translucency occurrence of pineapple fruit at harvest.

2. High fruit temperature during the last month of fruit development increases the occurrence of pineapple fruit translucency at harvest.

3. Spraying calcium during fruit development reduces the occurrence of pineapple fruit translucency at harvest.

4. Removal of crown during pineapple fruit development increases the occurrence of fruit translucency at harvest.

5. Removal of stem leaves to reduce the supply of photoassimilates at the latter stage of fruit development, without significantly affecting the fruit weight, reduces fruit flesh total soluble solids and the occurrence of pineapple fruit translucency at harvest.

6. Activity of cell-wall invertase is positively correlated to translucency occurrence in pineapple fruit flesh.

CHAPTER 4

EFFECTS OF FRUIT TEMPERATURE ON THE OCCURRENCE OF PINEAPPLE FRUIT TRANSLUCENCY

4.1 Introduction

The most significant effect of air temperature on pineapple fruit translucency occurs three months before harvest (Paull and Reyes, 1996). The significant correlations between the monthly average air temperature one and two months before harvest and fruit translucency are also negative (Paull and Reyes, 1996), though the translucency of flesh develops as the fruit matures and ripens. This result means that lower temperature during the early stage of fruit development produces more translucent fruits.

Air temperature alone is not an index of fruit temperature since on sunny days with about 100% exposure to sunlight the flesh temperature can be 54.5°C, while the maximum air temperature is only 22.8°C (Nightingale, 1942). Therefore, fruit temperature in spring could be significantly higher than those in summer, if the crown sizes are significantly smaller. Malézieux et al. (1994) reported that heat-units based on fruit temperature is more satisfactory than air temperature for predicting fruit development rate and harvest date.

Fruits with larger crowns have lower incidence and severity of translucency during the year (Paull and Reyes, 1996). The effect of crown size may be due to shading and reducing the fruit temperature in the daytime. High temperature can modify membrane composition and structure and cause ion leakage (Björkman et al., 1980; Quinn, 1988), suggesting that electrolyte leakage can be used as an indicator of high temperature injury (Chen at al., 1982; Inaba and Crandall, 1988).

The objectives of this study were to examine the effects of preharvest fruit temperature and postharvest heat treatment on electrolyte leakage and translucency occurrence of pineapple fruit flesh. Fruit shading or covering were used to alter fruit temperature in the daytime during development. Postharvest heat treatments were used to determine the effect of high temperature on the electrolyte leakage of fruit flesh cells at different fruit ages and whether it induced translucency.

4.2 Materials and methods

Plant material: Pineapple fruit (cv. 'Smooth Cayenne') with single crown and uniform size from the Dole Fresh Fruit Co. plantation on the island of Oahu in Hawai'i were used in all experiments in this research. The final date of fruit harvest coincided within one week of commercial harvest.

Determination of total soluble solids (TSS), titratable acidity (TA) and flesh translucency: TSS of fruit flesh juice was determined by refractometry. Acidity (TA) was determined by titration with 0.1 N NaOH, end point pH 8.2, using a Radiometer ABU 80 autoburette. Translucency severity of flesh was subjectively evaluated on a longitudinal cut half fruit, based on the percentage of affected area (0%: opaque, not translucent; to 100%: fully translucent) (Paull and Reyes, 1996). Various degrees of translucency severity are showed in Figure 4.1. Incidence of translucency was determined by computing the percentage of the affected fruit.

Determination of electrolyte leakage: Longitudinal flesh plugs were taken with a cork borer (10 mm diameter) and sliced into disks ca. 2 mm thick. About 6 g of disks were

washed three times with deionized water to remove lysed cell material. The disks were then gently shaken and incubated in 60 ml of 0.3 M mannitol solution for 2 hours. The conductivity of this solution was determined with a Radiometer CDM83 conductivity meter. The sample was then boiled for 2 hours to release all electrolytes and total conductivity measured. The electrolyte leakage was represented as the percentage of the total conductivity.

Translucency and electrolyte leakage: Six highly translucent and six opaque fruit were used to determined the electrolyte leakage of fruit flesh cells. The electrolyte leakage was determined by the method described above.

Postharvest heat treatment: This experiment was conducted from February 27, 1996 to May 07, 1996. Twelve fruit with uniform size were picked biweekly from 10 weeks before commercial harvest. Six fruit were kept at 22°C as the control, and the other six fruit were held in an incubator at 48°C, RH > 90% for 24 hours. The translucency severity and electrolyte leakage were then determined. Lower temperature (< 40°C) had no significant effect on inducing translucency and higher temperature (> 55°C) caused severe dehydration. It is important to keep the humidity in the incubator as high as possible during the heat treatment to prevent fruit from dehydration.

Fruit shading and covering in the last stage of development: Fruit, not including crown, were covered with clear plastic bag (0.1 mm thick) or shaded with opaque paper (0.3 mm thick) from three weeks before harvest. A randomized complete block design was used, and each treatment had 4 replications of 12 fruit each. TSS, TA and translucency were determined at harvest. This experiment was conducted from June 17, 1998 to July 08, 1998.

Fruit temperature was measured by inserting a thermocouple into the fruit 1cm beneath the shell and recorded by a datalogger.

Fruit shading and covering in early stage of development: This experiment was conducted from September 08, 1998 to November 27, 1998. Fruit, not including crown, were covered with clear plastic bag (0.1 mm thick) or shaded with opaque paper (0.3 mm thick) from 11 to 5 weeks before harvest. Half of the shaded fruit were then covered with clear plastic bag from 5 weeks before harvest to harvest. A randomized complete block design was used, and each treatment had 4 replications with 8 fruit each. Fruit weight, TSS, TA and translucency were determined at harvest.

To examine the direct effect of fruit temperature 3 to 2 months before harvest on the electrolyte leakage of fruit flesh, the electrolyte leakage in fruit covered with clear-plastic bag 11 weeks before harvest was determined at one and three weeks after treatments.

Lodged fruit: To examine the effect of high fruit temperature caused by high radiation on the flesh characteristics, twelve lodged fruit, with translucent flesh on the adaxial (exposed) side, were picked one week before harvest. Fruit flesh tissues were separated into two portions, adaxial (exposed-side) and abaxial (shaded-side). TSS and TA were determined.

4.3 Results

The electrolyte leakage was higher in highly translucent fruit than in opaque fruit (Table 4.2). The postharvest heat treatment (48°C, 24 hours) did not induce flesh translucency in fruit 10 to 6 weeks before harvest (Figure 4.2). However, it elevated

translucency thereafter. The electrolyte leakage in pineapple fruit flesh cells 10 and 8 weeks before harvest was reduced by the postharvest heat treatment (Table 4.2). The electrolyte leakage and the susceptibility to postharvest heat treatment increased during the last 4 weeks of fruit development (Figure 4.3).

Fruit temperature of clear-plastic-covered fruit averaged 3 to 4°C higher than the control between 10 and 12 a.m. in the exposed side of fruit flesh (1 cm below the shell), and the fruit temperature can be reduced as much as 10°C by shading. Fruit shaded during the last stage of development had the highest TA followed by the control and clear-plastic-covered fruit (Table 4.3). The TSS in shaded fruit was higher than that in the control and clear-plastic-covered fruit. The clear-plastic-covered fruit had higher translucency severity than the control and shaded fruit.

The fruit weight in the clear-plastic-covered fruit from 11 to 5 weeks before harvest was lower than the control (Table 4.4). Fruit shaded from 11 to 5 weeks before harvest followed by covering till harvest had the highest translucency severity. The electrolyte leakage of clear-plastic-covered fruit was significantly lower than the control, one and three weeks after treatment (Table 4.5).

Both the TSS and TA were significantly lower in the adaxial (exposed-side) portion of lodged fruit flesh than that in the abaxial (shaded-side) portion (Table 4.6). The TA of the adaxial portion was ca. 40% lower than the abaxial portion.

4.4 Discussion

Pineapple fruit translucency began to occur 4 to 2 weeks before harvest (Figure 4.2),

and followed a rapid increase in electrolyte leakage 6 to 4 weeks before harvest (Figure 4.3). This suggested that the electrolyte leakage of pineapple fruit flesh tissue increased with maturation, and translucency occurrence was coincided with an increase in electrolyte leakage. An increase in solute leakage indicating a loss of membrane integrity is very common during plant senescence and fruit ripening (Ferguson, 1984). Yamaki and Ino (1992) found that permeability to sugars across cell membranes increased in apple fruit with maturation. The electrolyte leakage in highly translucent pineapple fruit was higher than that in the opaque fruit (Table 4.1). Pineapple fruit translucency is similar to the watercore disorders in apples and pear (Marlow and Loescher, 1984), and an increase in membrane permeability was also found in watercore tissue of apple fruit (Kollas, 1968). Therefore, an increase in membrane permeability would favor the occurrence of pineapple fruit translucency. Covering fruit to raise fruit temperature from 3 or 5 weeks before commercial harvest increased the translucency severity of pineapple fruit at harvest (Table 4.3 and 4.5). It was also found that apple watercore increased with the increasing field fruit temperature (Fisher et al., 1930). In addition, postharvest heat treatment (48°C, 24 hours) increased translucency severity (Figure 4.2) and the electrolyte leakage in the pineapple fruit flesh (Figure 4.3) in the last 4 weeks of fruit development. This suggested that high fruit temperature during the last stage of pineapple fruit development would favor translucency occurrence, possibly through an effect on membrane permeability. However, an increase in membrane permeability may play only a minor role in translucency occurrence since the difference in electrolyte leakage between highly translucent fruit and opaque fruit was only 6% at harvest (Table 4.1). This

was consistent with the result reported by Soler (1994), which showed that the conductivity of translucent fruit flesh tissue was only ca. 4% higher than that of opaque fruit after 2 hour incubation in 0.3 M mannitol solution.

Fruit temperature (1 cm below the shell) of clear-plastic-covered fruit averaged 3 to 4°C higher than the control between 10 and 12 am in the exposed side of fruit flesh in a partly cloudy day, and the fruit temperature can be reduced as much as 10°C by shading with opaque paper. However, shading fruit during the last stage of fruit development did not reduce translucency occurrence (Table 4.3), while covering did increase the occurrence of translucency (Table 4.3 and 4.4). Similar results were also found in apples, that shading with cheesecloth did not prevent watercore (Kollas, 1968), while covering with black cambric and transparent cellophane increased watercore (Fisher et al., 1930).

Postharvest heat treatment reduced the electrolyte leakage in the pineapple fruit flesh tissues picked 10 and 8 weeks before commercial harvest (Table 4.2) and covering fruit with clear-plastic bag to raise fruit temperature during the daytime at young fruit stage also reduced the electrolyte leakage of fruit flesh cells (Table 4.5). The results suggested that during early pineapple fruit development, high daytime fruit temperature may increase the fruit heat tolerance, that would reduce the occurrence of heat stress induced fruit translucency during the last stage of fruit development. Plant cells have the ability to increase thermotolerance when grown for a long period at elevated temperature or when subjected to a transient heat stress (Santarius and Weis, 1988). Acclimation of cultured pear cells growing at elevated temperature led to a general increase in heat tolerance (Wu and Wallner, 1984). Apple fruit that had been previously exposed to high irradiation were more resistant to heat

stress than those had been partially protected (Brooks et al., 1926). Therefore, the significantly negative correlation between the air temperature 3 to 1 month before harvest and pineapple fruit translucency (Paull and Reyes, 1996) was possibly due to an increase in thermotolerance of fruit flesh, obtained from higher fruit temperature 3 to 1 month before harvest.

In general, translucent pineapple fruits usually have lower acidity (Sideris and Krauss, 1933b), and taste sweeter (Sideris and Krauss, 1934) than opaque fruits. Covering fruit with clear-plastic from 3 weeks before harvest decreased the severity of fruit translucency and TA at harvest (Table 4.3), implying that fruit temperature during the day in the last stage of fruit development may play a role in not only the translucency occurrence but fruit juice TA level at harvest. The adaxial (exposed) portion of lodged pineapple fruit had significant lower TA and TSS, but TSS-to-TA ratio was higher (Table 4.6). The results suggested that the higher sweetness of translucent pineapple fruit was at least partially due to the significantly reduced acidity caused by high fruit temperature during the last stage of fruit development.

The effects of fruit temperature on pineapple translucency occurrence can be divided into two stages, before and after 6 weeks before harvest, because high fruit temperature reduced membrane permeability of fruit flesh when fruit age was younger than 8 weeks before harvest, but increased it when fruit age was 4 weeks before harvest or older.

Table 4.1. Differences in electrolyte leakage between highly translucent and opaque pineapple fruit flesh. Data were analyzed as a completely randomized design.

Fruit types	Electrolyte leakage (%)
Highly translucent	72.7
Opaque	66.7
Analysis of variance F-test, probability	< 0.01
LSD 5%	3.4

Mean of 6 fruit, 2 measurements per fruit.

Table 4.2. Effects of postharvest heat treatment (48°C, 24 hours) on electrolyte leakage of pineapple flesh cells 10 and 8 weeks before harvest. Data were analyzed as a completely randomized design.

Treatments	Electrolyte leakage (%)	
	10 weeks before harvest	8 weeks before harvest
Heat treated fruit	9.4	14.1
Control	12.3	16.6
Analysis of variance F-test, probability	< 0.01	0.024
LSD 5%	1.7	2.1

Mean of 6 fruit, 2 measurements per fruit.

Table 4.3. Effects of covering or shading fruit in the last stage of development on pineapple fruit characteristics at harvest. Fruit were covered with clear plastic bag or shaded with opaque white paper from 3 weeks before harvest.

Treatments	TSS (%)	TA (meq/100 ml)	Translucency (%)	
			Severity	Incidence
Fruit covered	13.7 b	12.7 c	5.1 a	43.8
Fruit shaded	14.4 a	15.4 a	2.1 b	27.1
Control	13.7 b	14.1 b	3.0 b	33.3
Analysis of variance F-test, probability	0.035	< 0.01	< 0.01	0.057
LSD 5%	0.6	0.5	1.5	

Mean of 4 replicates, 12 fruit per replicate.
Means in the same column followed by the same letter were not significantly different at the 5% level by the LSD test.
Analysis of variance in the means of translucency severity and incidence was based on the square root and angular transformed data, respectively. Data presented here are the original untransformed means.
This experiment was conducted from June 17, 1998 to July 08, 1998.

Table 4.4. Effects of covering or shading fruit from 11 to 5 weeks before harvest on pineapple fruit characteristics at harvest. Fruit were covered with clear plastic bag or shaded with opaque white paper from 11 to 5 weeks before harvest; or shaded with opaque white paper from 11 to 5 weeks before harvest followed by covering with clear plastic bag till harvest.

Treatments	Fruit weight (g)	Crown weight (g)	TSS (%)	TA (meq/100ml)	Translucency (%)	
					Severity	Incidence
Covered fruit from 11 to 5 weeks before harvest	1337 b	151	15.2	16.2	5.9 b	56.3
Shaded fruit from 11 to 5 weeks before harvest	1415 ab	136	14.8	16.4	11.3 b	68.8
Shaded fruit from 11 to 5 weeks before harvest followed by covered fruit till harvest	1498 a	152	15.2	15.4	19.7 a	68.8
Control	1463 a	145	14.9	15.2	11.1 b	65.6
Analysis of variance F-test, probability	0.044	0.21	0.61	0.49	0.012	0.44
LSD 5%	111				7.4	

Mean of 4 replicates, 8 fruit per replicate.
Means in the same column followed by the same letter were not significantly different at the 5% level by the LSD test.
Analysis of variance in the means of translucency severity and incidence was based on the square root and angular transformed data, respectively. Data presented here are the original untransformed means.
This experiment was conducted from September 08, 1998 to November 27, 1998.

Table 4.5. Effects of covering fruit on the electrolyte leakage of pineapple fruit flesh cells. Fruit were covered with clear plastic bag from 11 to 10, or 11 to 8 weeks before commercial harvest. The electrolyte leakage of flesh cells was determined at end of treatments. Data were analyzed as a completely randomized design.

Treatments	Electrolyte leakage (%)	
	One week after treatment	Three weeks after treatment
Fruit covered	9.5	11.4
Control	10.9	13.2
Analysis of variance F-test, probability	0.022	0.029
LSD 5%	1.1	1.4

Mean of 4 fruit, 2 measurements per fruit.

Table 4.6. Differences in total soluble solids (TSS) and titratable acidity (TA) between adaxial and abaxial sides of lodged pineapple fruit flesh. Fruit were picked one week before harvest and cut longitudinally into two portions, adaxial and abaxial. Data were analyzed as a randomized complete block design.

Fruit portions	TSS (%)	TA (meq/100ml)	Ratio (TSS/TA)
Adaxial (exposed-side)	11.9	5.8	2.1
Abaxial (shaded-side)	13.2	9.5	1.4
Analysis of variance F-test, probability	< 0.01	< 0.01	< 0.01
LSD 5%	0.8	1.1	0.4

Mean of 12 fruit.

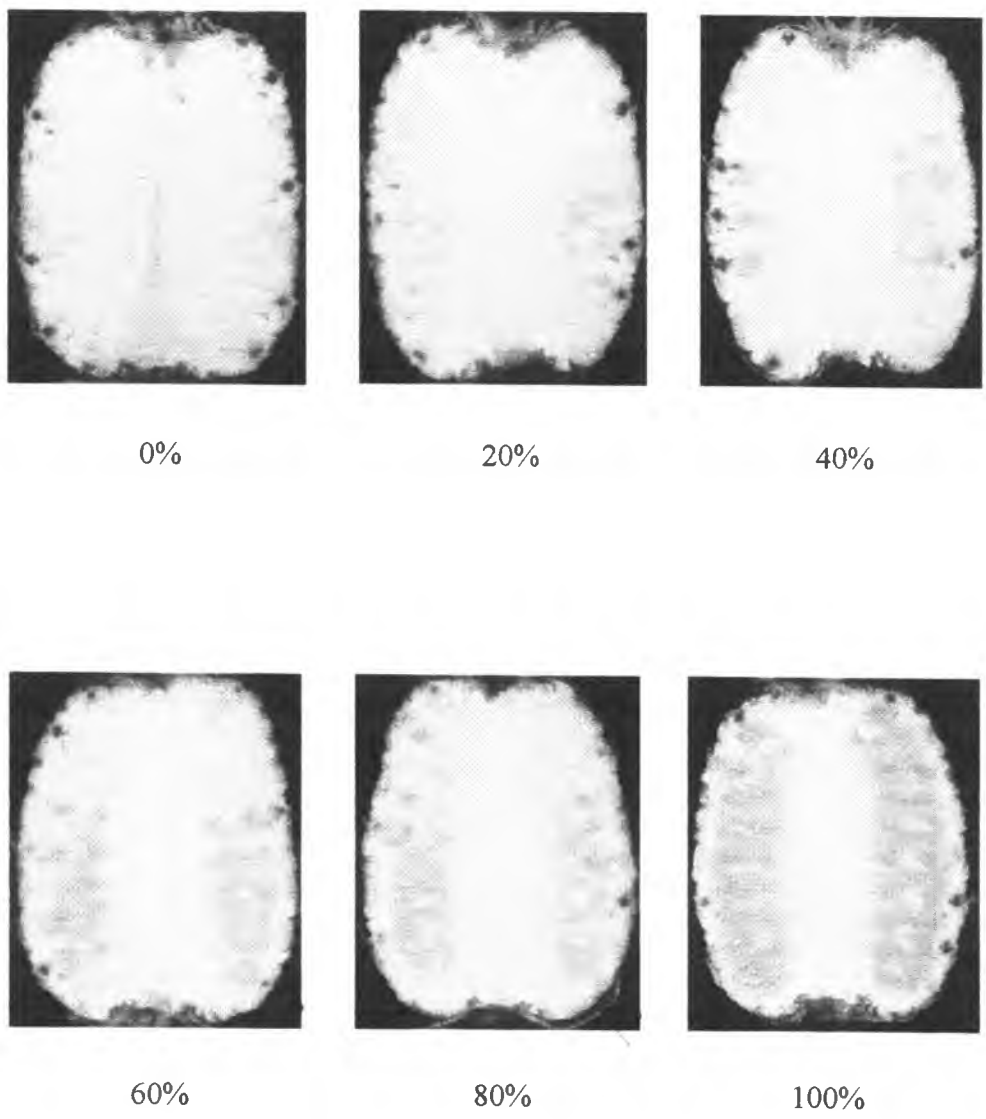


Figure 4.1. Various degrees of translucency severity.

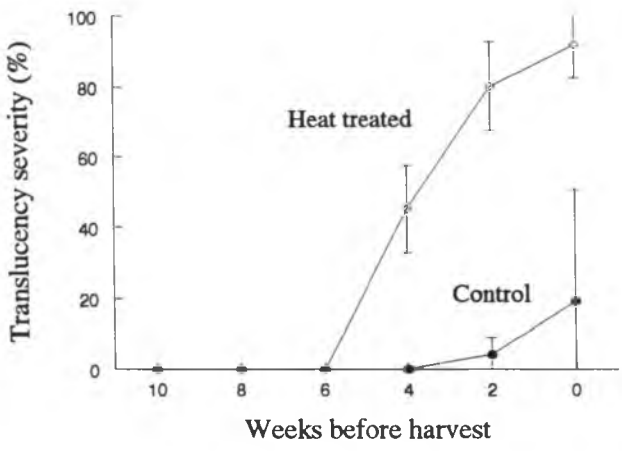


Figure 4.2. Pineapple fruit translucency induced by postharvest heat treatment (48°C, 24 hours) of fruit harvested every 2 weeks from 10 weeks before harvest. Control (●), heat treated (○). Mean ± SD of 6 fruit. This experiment was conducted from February 27, 1996 to May 07, 1996.

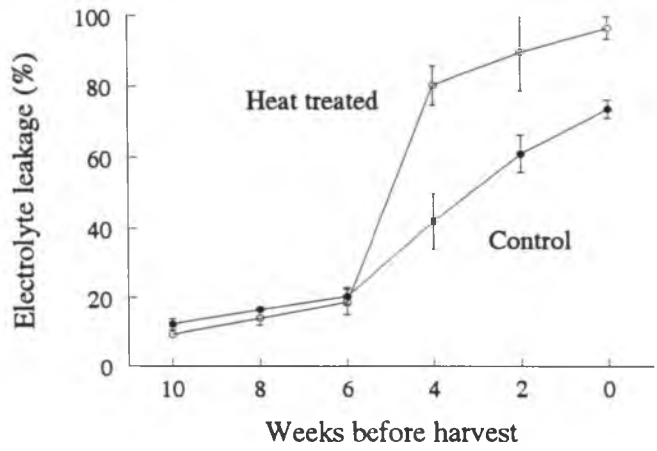


Figure 4.3. Electrolyte leakage of pineapple fruit flesh cells induced by postharvest heat treatment (48°C, 24 hours) of fruit harvested every 2 weeks from 10 weeks before harvest. Control (●), heat treated (○). Mean ± SD of 6 fruit, 2 measurements per fruit. This experiment was conducted from February 27, 1996 to May 07, 1996.

CHAPTER 5

EFFECTS OF CALCIUM ON THE OCCURRENCE OF PINEAPPLE FRUIT TRANSLUCENCY

5.1 Introduction

Calcium is related to many physiological disorders of fruit and vegetables (Shear, 1975). Calcium deficiency renders membranes more permeable (Simon, 1978) and could account for a loss of turgor and permit cell fluid to invade intercellular spaces. Calcium is an immobile macronutrient in plants (Hanger, 1979), and its movement is predominantly upwards from the roots and generally routed to meristematic zones and young tissues (Hanger, 1979). Calcium deficiency can cause a very small pineapple crown and death of terminal meristem when deficient is severe (Py et al., 1987). The import of calcium into fruit diminishes with fruit development, and virtually stops with the onset of the rapid intake of photosynthates via the phloem (Hanger, 1979).

Plant senescence and fruit ripening are significantly influenced by calcium. The major sites for the action of calcium are suggested to be the membrane structure and function, and cell wall structure (Ferguson, 1984). Membrane-associated calcium ions can reduce electrolyte leakage caused by stress (Poovaiah, 1993). Preharvest foliar application of calcium chloride delays postharvest ripening of strawberries (Chéour et al., 1990) and delays cabbage leaf senescence by protecting membrane lipids from degradation (Chéour et al., 1992).

Disorders caused by an inadequate calcium content in affected tissues cannot be corrected simply by supplying additional calcium to the soil (Shear, 1975), as there is no

guarantee that calcium supplied in the soil will be transported into the affected tissues. The entire system of uptake and translocation must be considered and the necessary adjustments made to assure adequate calcium accumulation in affected tissues (Shear, 1975).

Calcium sprays may supply sufficient calcium to control fruit disorders. However, the calcium from sprays must move into fruits through their surface, and only very limited quantities are supplied in this way (Shear, 1975). Therefore, for each fruit species, the time of spraying and calcium formulation are crucial.

Cell division in pineapple fruit is completed before flowering, and the increase in fruit size after flowering and during development results from the enlargement of existing cells (Collins, 1960). Pineapple fruit translucency increases with increasing fruit weight (Bowden, 1969), possibly due to a decrease in calcium concentration since larger fruit may need more calcium to stabilize cell membranes. When fruit cannot acquire sufficient calcium, cell membranes may lose integrity and lead to leakage and translucency.

The objectives of this study were to examine the changes in calcium content in fruit flesh during development and determine the effect of calcium sprays during fruit development on the occurrence of translucency at harvest.

5.2 Materials and methods

Plant material: Pineapple fruit (cv 'Smooth Cayenne') with single crown and uniform size from the Dole Fresh Fruit Co. plantation on the island of Oahu in Hawai'i were used in all experiments in this research. The final date of fruit harvest coincided within one week of commercial harvest.

Determination of calcium concentration: Fruit flesh tissue (50 g, sampled from pooled 3 fruit tissues) was homogenized in a blender with 100 ml deionized water. An aliquot (20 ml) was mixed with an equal volume of 12 M HCl and heated at 60°C in a water bath for 30 min. The solution was then filtered through a Whatman #42 ashless filter paper into a 50-ml volumetric flask and made up to the volume with deionized water. Calcium concentration was determined by an inductively coupled plasma analyzer (Perkin-Elmer, ICP/6500).

Calcium application and fruit development: Ten fruit were picked every 4 weeks from 12 weeks before harvest. Fruit flesh was separated into fruitlet and interfruitlet tissues. The concentration of calcium in each fruit flesh tissue was then determined.

Fruit were sprayed with 1% (w/v) $CaCl_2$ every two weeks from 12 , 8, or 4 weeks before harvest, respectively. Calcium solution (ca. 25 ml) was directly sprayed on each individual fruit, including the crown. The 12-week treatment had 6 sprays; 8 weeks, 4 sprays and 4 weeks, 2 sprays. A randomized complete block design was used. Each treatment had 4 replicates with 6 fruit each. Fruit weight, crown weight, TSS, TA and fruit translucency were determined at harvest as described in Chapter 4. This experiment was conducted from February 14, 1996 to May 08, 1996.

Flesh binding cations: To compare the differences in the divalent cation binding ability of cell walls at various ages of fruit flesh. Freehand cross sections from fruit 8 weeks before commercial harvest and fruit 1 week before commercial harvest were stained for Ni^{2+} binding as described by Varner and Taylor (1989). These sections were washed sequentially in 0.5 % Nonidet P-40 for 30 min, 20 % Clorox for 30 min, and 0.05 % Nonidet P-40 for 30 min. Samples were then placed in 10 mM $NiCl_2$ for 30 min followed by rinsing in 0.05 %

Nonidet P-40 for 30 min. After placing the prepared sections in deionized water, Na_2S was added to a final concentration of 0.2% until a dark brown to black color developed.

Apoplastic tracer movement: The apoplastic network in pineapple fruit flesh was studied using the apoplastic tracer amido black. The difference in apoplastic dye movement between fruit 8 weeks and 1 week before commercial harvest was compared. The freshly cut end of the fruit peduncle was placed in 0.1 % (w/v) amido black and the fruit maintained in a hood with continuous air flow for 24 hours. The dye distribution in the fruit flesh was then examined after the fruit was cut longitudinally.

Calcium formulation: To compare the effects of different calcium formulations on translucency occurrence, fruit were sprayed 3 times with 1 and 2% (w/v) $CaCl_2$ or 1 and 2 % (w/v) $Ca(NO_3)_2$ at 10, 8, and 6 weeks before commercial harvest. A randomized complete block design was used. Each treatment had 4 replicates with 8 fruit each. Fruit and crown weight, TSS, TA and fruit translucency were determined at harvest as described in Chapter 4. This experiment was conducted from March 27, 1997 to June 04, 1997.

Quantity of calcium: This experiment was conducted from April 14, 1998 to July 07, 1998. Fruit were sprayed 3 times with 2 to 8% (w/v) $CaCl_2$ at 12, 9 and 6 weeks before harvest. A randomized complete block design was used. Each treatment had 4 replicates with 12 fruit each. Flesh calcium concentration was determined at harvest.

5.3 Results

The concentration of calcium in the fruit flesh tissue declined dramatically from 12 weeks to 8 weeks before commercial harvest (Figure 5.1). The percentage of decrease in

calcium concentration was greater in the fruitlet than in the interfruitlet tissue, though the calcium concentration was initially higher in the fruitlet tissue.

There was no significant effect of any calcium spray treatments on fruit and crown weight, TSS or TA (Table 5.1). Spraying calcium did significantly reduce the severity of fruit translucency.

Young fruit exhibited high Ni^{2+} binding, whereas the mature fruit showed little Ni^{2+} binding as indicated by the amount of NiS black precipitates (Figure 5.2). The results suggest a significant reduction in flesh divalent cation binding capacity. Amido black after 24 hours of uptake was found throughout the young fruit flesh, and in mature fruit flesh the dye was only found in the core (Figure 5.3).

A $CaCl_2$ spray was more effective than $Ca(NO_3)_2$ in reducing translucency (Table 5.2). There was no significant difference between fruit and crown weight, TSS or TA between these two spray formulations.

There was a higher calcium concentration in the fruit flesh in the calcium treated fruit flesh than the control (Table 5.3). Calcium spray treatments of 6% and 8% $CaCl_2$, applied at the dry petal stage (12 weeks before commercial harvest), caused severe injury and 4% $CaCl_2$ caused slight to moderate injury to the crown leaves. The 2% $CaCl_2$ treatment caused slight injury on some crown leaf tips that was hardly noticeable after the crown grew larger. Larger and older crown leaves were more resistant to the $CaCl_2$ treatment than younger leaves. No injury was noticed on the fruit.

5.4 Discussion

Calcium spray treatments during fruit development reduced the occurrence of fruit translucency at harvest (Table 5.1 and 5.2). Calcium ions are involved in maintenance of middle lamella and membrane integrity (Leshem, 1992), and in the linkage of the pectic substances in the cell wall (Ferguson, 1984). Pineapple fruit translucency has been suggested to be related to an increase in cell wall hydrolases (Soler, 1993), and membrane permeability (Soler, 1994). High calcium concentration has been suggested to decrease cell wall hydrolase secretion or activities (Huber, 1983). As a result, increasing the calcium concentration in the fruit flesh may reduce the occurrence of pineapple fruit translucency.

The calcium concentration in pineapple fruit flesh declined with development (Figure 5.1), which was consistent with the results found in apple (Wiersum, 1966; Ferguson and Watkins, 1989) and tomato (Ho et al., 1987). The biggest decrease in calcium content in pineapple fruit flesh occurred after flowering, 12 to 8 weeks before harvest (Figure 5.1), suggesting that this period was a proper time to apply calcium. In addition, the floral cavity in the center of the eyes is still open (Kerns et al., 1936), which may help the movement of sprayed calcium into the flesh tissues. The tolerance limit of pineapple fruit to $CaCl_2$ treatment was ca. 2% (w/v), since the treatment of 2% $CaCl_2$ caused only slight injury to some crown leaf tips at the dry petal stage, that was barely noticeable after the crown grew larger.

Spraying $CaCl_2$ was more effective than $Ca(NO_3)_2$ in reducing pineapple fruit translucency (Table 5.2). Nevertheless, $CaCl_2$ was also more likely to cause injury to the crown leaves than $Ca(NO_3)_2$. Therefore, a calcium formulation that can increase the calcium

uptake by the pineapple fruit flesh without the risk of injury to the crown leaves will be needed.

In pineapple fruit 8 weeks before commercial harvest, amino black solution (0.1%, w/v), an apoplastic tracer, was found throughout the whole fruit flesh. The dye was only found in the core near maturity (1 weeks before harvest) (Figure 5.3). These results suggest that a transition of the primary water movement pathway from the xylem to the phloem in the fruit may occur, that possibly accompanied a rapid increase in sucrose accumulation in the fruit flesh. It was found that during tomato fruit growth and development, the daily calcium accumulation rates decreased with the decline in the proportion of water import from xylem (Ho et al., 1987). The ability to bind divalent cation in pineapple fruit flesh also declined between the young (8 weeks before harvest) and mature fruit stage (1 weeks before harvest) (Figure 5.2). Therefore, not only did the total calcium concentration in pineapple fruit decline during development but cell wall binding capacity also declined.

Table 5.1. Effects of calcium sprays during fruit development on pineapple fruit characteristics at harvest. Calcium (1% $CaCl_2$) was sprayed every two weeks from 12, 8, or 4 weeks before commercial harvest.

Treatment (weeks before harvest)	Fruit weight (g)	Crown weight (g)	TSS (%)		TA (meq/100 ml)		Translucency (%)	
			Top	Bottom	Top	Bottom	Severity	Incidence
12	1106	188	15.0	16.2	17.6	16.6	1.0 b	21.0
8	1148	176	15.0	16.4	17.2	16.0	1.7 b	25.0
4	1172	169	14.3	15.9	17.0	16.2	6.5 ab	45.8
0	1285	183	14.2	15.8	17.9	17.3	9.0 a	49.8
Analysis of variance F-test, probability	0.16	0.64	0.28	0.62	0.15	0.11	0.037	0.29
LSD 5%							6.0	

Mean of 4 replicates, 6 fruit per replicate.
Means in the same column followed by the same letter were not significantly different at the 5% level by the LSD test.
Analysis of variance in the means of translucency severity and incidence was based on the square root and angular transformed data, respectively. Data presented here are the original untransformed mean.
This experiment was conducted from February 14, 1996 to May 08, 1996.

Table 5.2. Effects of calcium sprays during fruit development on pineapple fruit characteristics at harvest. Fruit were sprayed three times with 1, 2% (w/v) $CaCl_2$ or 1, 2% $Na(NO_3)_2$ at 10, 8 and 6 weeks before commercial harvest.

Treatments	Fruit weight (g)	Crown weight (g)	TSS (%)	TA (meq/100ml)	Translucency (%)	
					Severity	Incidence
$CaCl_2$, 1%	1228	182	14.7	10.3	1.4 bc	28.1 bc
2%	1214	178	14.6	10.2	0.8 c	12.5 c
$Ca(NO_3)_2$, 1%	1288	181	15.0	10.9	2.3 b	34.4 b
2%	1213	193	14.9	10.5	2.0 bc	37.5 b
Control	1250	188	15.0	10.4	5.0 a	62.5 a
Analysis of variance F-test, probability	0.70	0.81	0.80	0.32	< 0.01	< 0.01
LSD 5%					1.2	19.2

Mean of 4 replicates, 8 fruit per replicate.
Means in the same column followed by the same letter were not significantly different at the 5% level by the LSD test.
Analysis of variance in the means of translucency severity and incidence was based on the square root and angular transformed data, respectively. Data presented here are the original untransformed mean.
This experiment was conducted from March 27, 1997 to June 04, 1997.

Table 5.3. Effects of calcium sprays in different concentrations during fruit development on the calcium concentration in pineapple fruit flesh at harvest. Fruit were sprayed 3 times with $CaCl_2$ at 12, 9, and 6 weeks before commercial harvest.

Treatments (% (w/v) $CaCl_2$)	Calcium concentration ($\mu g\ g^{-1}$ FW)
8	147 a
6	145 a
4	120 b
2	112 bc
0	95 c
Analysis of variance F-test, probability	< 0.01
LSD 5%	22

Mean of 4 replicates.
Means in the same column followed by the same letter were not significantly different at the 5% level by the LSD test.
This experiment was conducted from April 14, 1998 to July 07, 1998.

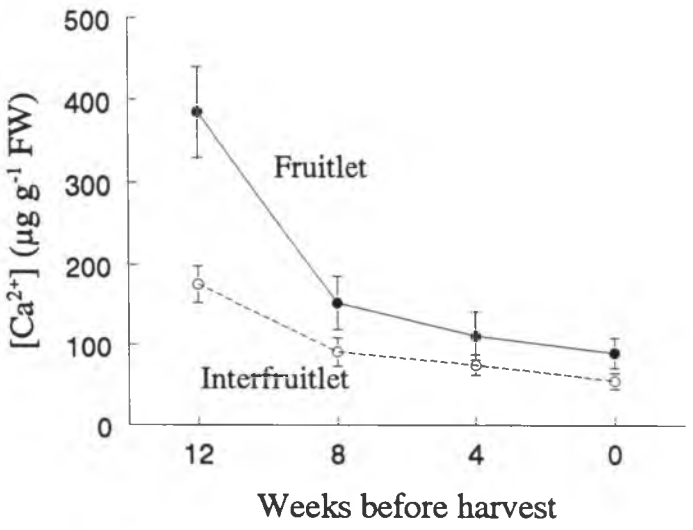


Figure 5.1. Changes in calcium concentration in pineapple fruit flesh during development. Fruit were picked every 4 weeks from 12 weeks before harvest, and fruit flesh was separated into fruitlet (●) and interfruitlet (○) tissues. Mean ± SD of 10 fruit.

Young tissue
(8 weeks before harvest)

Mature tissue
(1 week before harvest)

Figure 5.2. *In situ* localization of divalent cation binding at various ages of pineapple fruit flesh. Fruit sections were stained with NiS after allowing the Ni^{+2} to bind to the cell wall.

Young tissue
(8 weeks before harvest)

Mature tissue
(1 week before harvest)

Figure 5.3. Movement from the cut end of the peduncle of the apoplastic tracer amido black in fruit flesh of pineapple at various ages. Fruit were allowed to take up the dye for 24 hours before sectioning.

CHAPTER 6

EFFECTS OF REMOVING CROWN OR LEAVES ON THE OCCURRENCE OF PINEAPPLE FRUIT TRANSLUCENCY

6.1 Introduction

The correlation between the monthly average air temperature at 3 to 1 month before harvest and the incidence and severity of pineapple fruit translucency at harvest is significantly negative (Paull and Reyes, 1996). Crown weight is negatively correlated to pineapple fruit translucency during the year, and the correlation between crown weight and air temperature at 4 to 2 months before harvest is significantly positive (Paull and Reyes, 1996). These relationships suggest that the crown size may play a role in the occurrence of translucency. Since the fruit is a stronger sink than the crown during growth, the effect of crown on fruit translucency might be simply shading of the fruit and reduction of the fruit temperature during the day, or the crown may have no effect on the occurrence of translucency. Alternatively, both crown weight and fruit translucency could be affected by the same factor, air temperature, and there may be no cause-effect relationship between crown weight and fruit translucency.

Fruits forced by ethephon during cool nights have higher fruitlet numbers (Min and Bartholomew, 1997) , that may increase fruit growth, and reduce crown growth. Therefore, fruits harvested in spring have significantly small crowns (Paull and Reyes, 1996), due possibly in part to cool night temperature during the forcing treatment, producing more fruitlets.

Sideris and Krauss (1936) studied the effect of defoliation during pineapple fruit development and found that defoliation caused smaller fruit, lower sugar content and higher acidity. The effects of defoliation on fruit translucency were variable and no satisfactory conclusion could be drawn. One reason for this variability was possibly because plants were not forced into flowering so fruits in this trial were not harvested on the same date. There was a 1 to 3 week difference in harvest dates among the different treatments. Defoliation conducted at the red-bud stage led to a significant reduction in fruit translucency. However, the same treatment also significantly reduced fruit weight. Therefore, the reduction in translucency possibly was due to a delay in fruit growth rather than a direct effect of shortage of photoassimilate supply. To determine if there is a direct effect of photoassimilate on the occurrence of pineapple fruit translucency and without affecting fruit growth, defoliation needs be conducted at a later stage of fruit development, after the fruit has grown to full size.

The objectives of this study were to examine the effects of crown removal at an early and late stage of fruit development and stem leaf defoliation at the latter stages of fruit development on the occurrence of pineapple fruit translucency at harvest.

6.2 Materials and methods

Plant material: Pineapple fruit (cv. 'Smooth Cayenne') with single crown and uniform size from the Dole Fresh Fruit Co. plantation on the island of Oahu in Hawai'i were used in all experiments in this research. The final date of fruit harvest coincided within one week of commercial harvest.

Crown removal: This experiment was conducted from August 26, 1996 to October 21, 1996, when crowns were removed with a knife 10 weeks or 2 weeks before harvest. A randomized complete block design was used in this trial. Each treatment had four replicates with 8 fruit each. Fruit weight, total soluble solids (TSS), titratable acidity (TA), and fruit translucency were determined at harvest as described in Chapter 4.

Leaf defoliation: The first experiment was conducted from May 06, 1997 to June 04, 1997. Plants were defoliated at a late stage of fruit development, 4 weeks before commercial harvest, to avoid a significant effect on fruit weight. Total stem leaves were counted and 50 or 75% of individual leaves removed from bottom older ones upward. Each treatment had 4 replicates with 8 fruit each. Fruit weight, TSS, TA and fruit translucency were determined at harvest as described in Chapter 4.

The second experiment was conducted from December 08, 1998 to December 28, 1998. Plants were defoliated at a late stage of fruit development, 3 weeks before harvest, to avoid a significant effect on fruit weight. Total stem leaves were counted and 33.3, 66.7 or 100% of individual leaves removed from top younger ones downward. Each treatment had 4 replicates with 8 fruit each. Fruit weight, TSS, TA and fruit translucency were determined at harvest as described in Chapter 4.

6.3 Results

Removing the crown either at 10 weeks or 2 weeks before harvest did not cause any significant difference in fruit weight, TSS, TA, and fruit translucency at harvest (Table 6.1).

Fruit weight was not significantly affected by either defoliation (Table 6.2 and 6.3). The fruit TSS was significantly reduced by defoliation as were both translucency severity and incidence. The severity of translucency was reduced nineteenfold by 75% defoliation 4 weeks before harvest (Table 6.2) and 100% defoliation 3 weeks before harvest (Table 6.3). There appeared to be a cause-effect relationship between defoliation and TSS and translucency (Table 6.4). The significant correlation between TSS and translucency was positive (Table 6.5). The membrane permeability of pineapple fruit flesh cells was not significantly affected by defoliation (Table 6.6).

6.4 Discussion

Crown removal 10 or 2 weeks before harvest did not cause any significant effect on pineapple fruit weight and translucency (Table 6.1), suggesting that the crown did not have a significant influence on fruit growth and development, as suggested by Paull and Reyes (1996). Since both crown weight and translucency are correlated to air temperature 3 to 2 months before harvest (Paull and Reyes, 1996), it is likely that the significant correlation between crown weight and translucency is due to a third factor, air temperature, and there is no direct cause-effect relationship between crown weight and fruit translucency. In general, pineapple fruit translucency occurs more frequently in large fruit (Bowden, 1969). Fertilization during pineapple fruit development increases fruit weight and translucency, but reduces crown weight (Linford, 1934b). Applying chlorflurenol to reduce crown size did not affect the fruit size (Keetch and Dalldorf, 1980; Dalldorf, 1981). In contrast, fruit size may affect crown size since larger fruit generally have smaller crown and vice versa, suggesting

that pineapple fruit is a stronger sink than the crown during fruit development. This is consistent with the general observation that developing fruit are stronger sinks than vegetative organs for assimilates (Ho, 1988). As a result, it seems that crown size does not play a role in the occurrence of pineapple fruit translucency, since the crown is a weaker sink for assimilates than fruit, and the shading effect of crown on reducing fruit temperature during daytime is possibly also very limited.

Defoliation conducted in the early stage of pineapple fruit development led to smaller fruit, lower sugar content and higher acidity (Sideris and Krauss, 1936), suggesting leaf removal was delaying fruit growth. Since translucency generally begins during the last month of fruit development after the fruit has reached full size, the effects of defoliation on translucency need to be considered during this period. An increase in carbon unloading in the fruit flesh could lead to an increase in phloem sap movement into the fruit. This increased movement will , in turn, enhance water movement into the fruit flesh due to an increase in the osmotic pressure contributed by the imported solutes. The higher osmotic pressure and water uptake could favor the occurrence of translucency.

Defoliation did not significantly affect the membrane permeability of pineapple fruit flesh cells (Table 6.6). This suggested that translucency caused by carbon unloading is different from translucency caused by heat stress. Covering fruit in the last stage of pineapple fruit development increased translucency severity only 70% and had no significant effect on translucency incidence (Table 4.3), but both translucency severity and incidence were reduced fourfold and twofold respectively by 33.3% defoliation 3 weeks before harvest (Table 6.3). In addition, the translucency severity and incidence was reduced nineteenfold and fifteenfold

respectively by 75% defoliation 4 weeks before harvest (Table 6.2), and reduced nineteenfold and elevenfold respectively by 100% defoliation 3 weeks before harvest (Table 6.3). The results suggested that the effect of carbon unloading was much more significant than high fruit temperature in the last stage of pineapple fruit development on the translucency occurrence.

Table 6.1. Effects of crown removal during fruit development on pineapple fruit characteristics at harvest. Crowns were removed 10 or 2 weeks before harvest, respectively.

Treatments (weeks before harvest)	Fruit weight (g)	TSS (%)	TA (meq/100ml)	Translucency (%)	
				Severity	Incidence
10	1775	11.5	14.6	22.0	90.8
2	1766	12.0	14.8	22.7	87.8
Control	1692	11.8	13.6	24.7	87.5
Analysis of variance F-test, probability	0.36	0.45	0.18	0.88	0.89

Mean of 4 replicates, 8 fruit per replicate.
Analysis of variance in the means of translucency severity and incidence was based on the square root and angular transformed data, respectively. Data presented here are the original untransformed mean.
This experiment was conducted from August 26, 1996 to October 21, 1996

Table 6.2. Effects of defoliation during fruit development on pineapple fruit characteristics at harvest. Removal of 50 or 75% of the basal older stem leaves was conducted 4 weeks before harvest.

Treatments	Fruit weight (g)	Crown weight (g)	TSS (%)	TA (meq/100ml)	Translucency (%)	
					Severity	Incidence
75% defoliation	1097	163	11.8 c	11.3	0.2 b	3.1 b
50% defoliation	1102	165	13.2 b	10.7	0.5 b	9.4 b
Control	1175	176	14.8 a	10.6	3.8 a	46.9 a
Analysis of variance F-test, probability	0.39	0.60	< 0.01	0.25	< 0.01	< 0.01
LSD 5%			0.9		2.5	16.1

Mean of 4 replicates, 8 fruit per replicate.
Means in the same column followed by the same letter were not significantly different at the 5% level by the LSD test.
Analysis of variance in the means of translucency severity and incidence was based on the square root and angular transformed data, respectively. Data presented here are the original untransformed mean.
This experiment was conducted from May 06, 1997 to June 04, 1997.

Table 6.3. Effects of defoliation during fruit development on pineapple fruit characteristics at harvest. Removal of 33.3 to 100% of the stem leaves was conducted 3 weeks before harvest.

Treatments	Fruit weight (g)	Crown weight (g)	TSS (%)	TA (meq/100ml)	Translucency(%)	
					Severity	Incidence
100% defoliation	1416	183	8.4 d	12.2 a	0.47 b	6.3 c
66.7% defoliation	1443	170	9.3 c	11.7 ab	0.63 b	12.5 bc
33.3% defoliation	1463	176	10.2 b	11.7 ab	2.3 b	34.4 b
Control	1495	183	11.2 a	10.9 b	8.9 a	71.9 a
Analysis of variance F-test, probability	0.203	0.679	< 0.01	0.048	< 0.01	< 0.01
LSD 5%			0.6	0.8	3.1	23.1

Mean of 4 replicates, 8 fruit per replicate.
Means in the same column followed by the same letter were not significantly different at the 5% level by the LSD test.
Analysis of variance in the means of translucency severity and incidence was based on the square root and angular transformed data, respectively. Data presented here are the original untransformed mean.
This experiment was conducted from December 08, 1998 to December 28, 1998.

Table 6.4 Linear regression analysis of relationships between percentage of defoliation (D) 3 weeks before harvest and total soluble solids (TSS) and translucency in pineapple fruit at harvest.

	R^2	Probability > F	Equation
Total soluble solids (TSS)	0.91	< 0.01	TSS= 11.21 - 0.029D
Translucency severity (TS)	0.70	< 0.01	TS = 2.69 - 0.021D
Translucency incidence (TI)	0.71	< 0.01	TI = 54.14 - 0.490D

Table 6.5. Pearson correlations between total soluble solids and translucency in pineapple fruit at harvest. Correlation was done using defoliation data.

	Pearson correlation coefficient	Probability
Translucency severity	0.813	< 0.001
Translucency incidence	0.795	< 0.001

Table 6.6. Linear regression analysis of the relationship between percentage of defoliation (D) 3 weeks before harvest and electrolyte leakage of pineapple fruit flesh cells at harvest.

	R^2	Probability > F	Equation
Electrolyte leakage (EL)	0.116	0.104	EL = 66.44 - 0.064D

CHAPTER 7

CHANGES IN SUGAR CONTENT AND ACTIVITIES OF SUGAR METABOLIZING ENZYMES IN PINEAPPLE FRUIT FLESH DURING DEVELOPMENT

7.1 Introduction

Sugar content plays an important role in the flavor characteristics and commercial assessment of pineapple fruit quality (Py et al., 1987). Total soluble solids, mainly sugars, is often used as an indicator of fruit maturity and quality (Paull, 1993). Even though the commercial importance of the sugar content in pineapple fruit is recognized, our knowledge of carbohydrate metabolism in this fruit is limited.

Different pathways are used for sugar accumulation in various fruits (Hubbard et al., 1991), with different enzymes involved, including acid invertase (AI), sucrose synthase (SS), and sucrose-phosphate synthase (SPS) (Yamaki, 1995). The relationship between sugar composition and activities of sugar metabolizing enzymes in pineapple fruit has not been previously reported. In addition, the relationship between sugar composition and translucency is also obscure. Reduced assimilate supply caused by leaf defoliation at the latter stages of fruit development significantly reduced translucency severity and incidence (Table 6.2 and 6.3).

The objectives of this study were to determine the changes in the sugar composition and to relate these changes to the activities of sugar metabolizing enzymes during pineapple fruit development, and also to explore any correlation between the sugar accumulation and the occurrence of fruit translucency at the latter stages of fruit development.

7.2 Materials and methods

Plant material: Pineapple fruit (cv. 'Smooth Cayenne') with single crown and uniform size from the Dole Fresh Fruit Co. plantation on the island of Oahu in Hawai'i were used in this experiment. The final date of fruit harvest coincided within one week of commercial harvest. Fruit were picked biweekly from 12 weeks before commercial harvest. There were two sets of samples, each set had 12 fruit at each sampling date. One set was used for flesh analysis, the other was used for fruitlet and interfruitlet tissue analysis.

Tissue sampling: Flesh tissues from 4 fruit were diced and pooled together as one of three replicates at each sampling date. Tissues were immediately frozen in liquid nitrogen after sampling and stored at -20°C.

Carbohydrate analysis: Soluble sugars were extracted by the procedure described by Paull et al. (1984). Fruit flesh tissue (2 g) was heated to 90°C in a microwave oven to inactivate the enzymes, then homogenized with 18 ml 90% (v/v) ethanol. After standing for at least 24 hours at 1°C, an aliquot (5 ml) of the clear supernatant was taken to dryness under a stream of nitrogen and redissolved in 5 ml of deionized water. Quantities of individual sugars were determined by injecting an aliquot (20 μl) of sample, filtered through a 0.45 μm filter, and separated by HPLC equipped with a Bio-Rad Fast carbohydrate analysis column (100 x 7.8 mm) and refractive index detector. The mobile phase was degassed deionized water, at a flow rate of 0.6 ml min^{-1} at 80°C. Peak height measurements were used to quantify the soluble sugars expressed relative to standard solution.

Enzyme extraction: Soluble enzymes (SS, SPS, AI & NI) were extracted by the method described by Hubbard et al. (1991), with some modifications. Frozen tissues were

ground to a powder in liquid nitrogen, using a chilled mortar and pestle with a 1:4 tissue-to-buffer ratio. The buffer contained 100 mM Mops-NaOH (pH7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, 0.05% (v/v) Triton X-100, 10 mM ascorbic acid, 1 mM PMSF, 20 μM E-64, 1 mg ml^{-1} BSA, and 10 mg ml^{-1} PVPP. The homogenate was filtered through 8 layers of cheesecloth and centrifuged at 20,000x g for 20 min. The supernatant was desalted with an Amicon CF25 membrane cone. The desalting buffer contained 100 mM Mops-NaOH (pH 7.5), 5 mM $MgCl_2$ and 2.5 mM DTT.

For cell-wall invertase (CWI) extraction, the homogenate, without filtering through cheesecloth, was centrifuged at 20,000x g for 30min. After centrifugation, the supernatant was discarded, and the pellet washed with deionized water and centrifuged again at 20,000x g for 30 min. The pellet was then suspended in 10 ml 100 mM citrate-phosphate buffer (pH 4.5) containing 2.5 mM DTT, 1 M NaCl and 1 mg ml^{-1} BSA. The suspension was kept at 4°C for 24 hours. The extract was then centrifuged at 20,000x g for 30 min. The supernatant was desalted as described above. The desalting buffer contained 100 mM citrate-phosphate (pH 4.5), 5 mM $MgCl_2$ and 2.5 mM DTT.

Enzyme assay: SPS activity was assayed by the procedure described by Hubbard et al. (1989), with some modifications. Reaction mixtures (100 μl) contained 100 mM Mops-NaOH (pH 7.5), 15 mM $MgCl_2$, 10 mM fructose-6-P, 50 mM glucose-6-P, 10 mM UDPG, and 50 μl desalted extract. Reaction mixtures were incubated at 35°C and terminated at 0 and 30 min with 100 μl of 30%(w/v) KOH, followed by placing the tubes in boiling water for 10 min to destroy any unreacted reducing sugars. After cooling, 2 ml of a mixture of 0.15% (w/v) anthrone in 13.8 M H_2SO_4 was added and incubated in a 40°C water bath for 20 min.

cooling, color development was measured at 620nm. SS assay was measured in sucrose synthesis direction and was identical to that of SPS except the reaction mixtures contained 20 mM fructose without fructose-6-P or glucose-6-P.

Reaction mixtures (100 μl) to determine NI activity contained 100 mM Mops-NaOH (pH 7.5), 100 mM sucrose, and 50 μl desalted extract. The mixtures were incubated at 35°C and terminated at 0 and 30 min by placing the tubes into boiling water for 3 min. Hexose sugar concentration was determined by the method described by Honda et al. (1982) with slight modifications. After the reaction was terminated, 1.0 ml of cold 100 mM borate buffer (pH 9.0) was added, followed by 0.1 ml of 2-cyanoacetamide. Tubes were then placed in a boiling water bath for 10 min. After cooling, the absorbance at 276 nm was determined. Measurements of reducing sugars were expressed relative to standard solution containing equal concentrations of glucose and fructose. The procedure for AI and CWI assay was identical to that of NI except the reaction buffer was 100 mM citrate-phosphate (pH 4.5) and the reaction was terminated by directly adding 1.0 ml of cold 100mM borate buffer (pH 9.0) instead of heating to prevent acid breakdown of sucrose.

7.3 Results

During the early stages of pineapple fruit development, total soluble sugar content was low and composed mainly of glucose and fructose, with glucose at a slightly higher concentration than fructose (Figure 7.1). Sucrose accumulated rapidly 6 weeks before harvest and ultimately exceeded the glucose and fructose concentration (Figure 7.1). Sucrose accumulated more in the fruitlet than in the interfruitlet tissue (Figure 7.2) until the last 2

weeks of fruit development, when sucrose accumulation rate in the interfruitlet tissue was greater than in the fruitlet. Though glucose and fructose remained relatively constant throughout development, there was a slight increase in fructose in the last 2 weeks of fruit development (Figure 7.1).

SS activity was high in younger fruit and declined to a very low level, while SPS activity was relatively low and constant throughout fruit development (Figure 7.3). The activities of all three invertases were higher in younger fruit and declined to low levels 6 weeks before harvest (Figure 7.4). CWI activity increased again 4 weeks before harvest, while the activities of NI and AI remained low (Figure 7.4).

7.4 Discussion

During the early stage of pineapple fruit development, the glucose and fructose were the predominant sugars (Figure 7.1) and the activities of SS and invertases were high, the activity declined to very low level 6 weeks before harvest when sucrose started to accumulate (Figure 7.3 and 7.4). This agrees with the general observation that glucose and fructose are the predominant sugars in rapidly growing tissues, which often have high invertase activity (Morris and Arthur, 1984). It has been found that activities of sucrose hydrolyzing enzymes (SS and invertases) are high in young fruit and declined with fruit development concomitantly with sucrose accumulation (Lingle and Dunlap, 1987; Islam et al., 1996; Vizzotto et al., 1996). There was a positive correlation between sucrose accumulation and translucency occurrence, because sucrose began to rapidly accumulate 6 weeks before harvest (Figure 7.1), followed by the occurrence of translucency 4 to 2 weeks before harvest (Figure 4.2). In

general, the fruitlet show the translucency symptom earlier than the interfruitlet tissues and the fruitlet accumulated sucrose ca. 2 weeks earlier than the interfruitlet tissue (Figure 7.2). This implied that the fruitlet was 2 weeks ahead of the interfruitlet tissue in development.

In some fruit species, SPS catalyzes sucrose synthesis (Yamaki, 1995). Though SPS activity did not increase during pineapple fruit development (Figure 7.3), sucrose still accumulated in the last 6 weeks of fruit development while the activities of sucrose hydrolyzing enzymes (SS, AI and NI) were low. The activities of SS, NI and AI declined with pineapple fruit maturation, concomitant with the accumulation of sucrose, indicating that activities of these enzymes were important in determining the composition of stored soluble sugars in pineapple fruit flesh.

The long-distance transport of carbohydrates, mostly sucrose, between source and sink, occurs in the phloem and is driven by the pressure gradient generated by loading of photoassimilate at the source and unloading at the sink organs (van Bel, 1992a). Since the removal of sucrose increases the pressure gradient and enhances the flow toward sinks, enzymes involved in sucrose metabolism are expected to be important both for phloem unloading and for the import of sucrose into sink organs. Therefore, the high CWI activity, favoring apoplastic unloading, at the last stage of pineapple fruit development (Figure 7.4) may be responsible for maintaining a pressure gradient between the source and sink. In addition, a high CWI activity may increase the solute concentration and osmotic pressure and thereby the liquid volume in the apoplast, that in turn reduces the intercellular air spaces and leads to the occurrence of translucency.

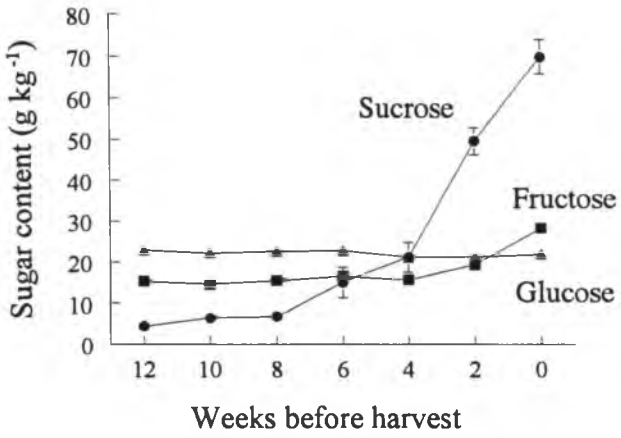


Figure 7.1. Changes in sucrose (●), glucose (▲), and fructose (■) contents of developing pineapple fruit flesh. Fruit were picked biweekly from 12 weeks before harvest. Mean ± SD of three replicates, 2 measurements per replicate.

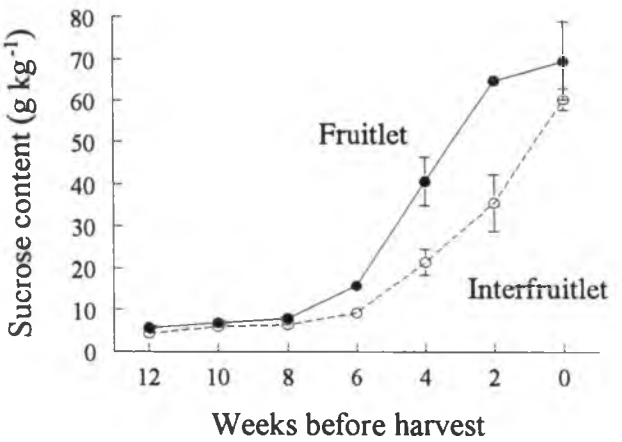


Figure 7.2. Differences in sucrose content between fruitlet (●) and interfruitlet (○) tissues during pineapple fruit development. Fruit were picked biweekly from 12 weeks before harvest. Mean ± SD of three replicates, 2 measurements per replicate.

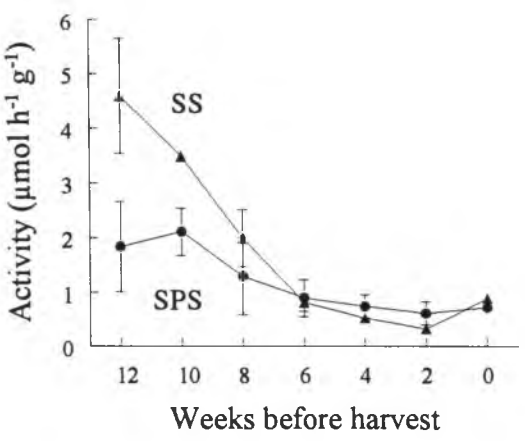


Figure 7.3. Changes in the activities of sucrose phosphate synthase (SPS) (●) and sucrose synthase (SS) (▲) in developing pineapple fruit flesh. Fruit were picked biweekly from 12 weeks before harvest. Mean ± SD of three replicates.

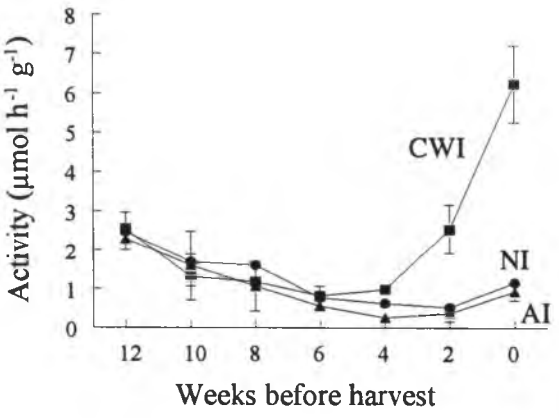


Figure 7.4. Changes in the activities of acid invertase (AI) (●), neutral invertase (NI) (▲), and cell-wall invertase (CWI) (■) in developing pineapple fruit flesh. Fruit were picked biweekly from 12 weeks before harvest. Mean ± SD of three replicates.

CHAPTER 8

THE RELATIONSHIPS BETWEEN THE ACTIVITY OF CELL WALL INVERTASE AND TRANSLUCENCY IN PINEAPPLE FRUIT FLESH

8.1 Introduction

The plant invertases, acid invertase (AI), neutral invertase (NI) and cell-wall invertase (CWI), have been suggested to play important biological functions in source/sink relationships, phloem loading and unloading, growth and development (Tymowska-Lalanne and Kreis, 1998). The role of CWI in apoplastic phloem unloading, cleaving unloaded sucrose into glucose and fructose to maintain the pressure gradient between source and sink has been recognized (Roitsch et al., 1995).

The intercellular free spaces are filled with liquid in translucent pineapple fruit flesh tissues, suggesting that a higher osmotic pressure exists in the apoplast that steepens the pressure gradient between the phloem end and the sink. Therefore, the high activity of CWI in the pineapple fruit flesh could be a positive factor in the occurrence of translucency. The objective of this study was to determine if there was a significant correlation between the CWI activity and the severity of translucency in pineapple fruit flesh.

8.2 Materials and methods

Plant material: Pineapple fruit (cv. 'Smooth Cayenne') with single crown and uniform size from the Dole Fresh Fruit Co. plantation on the island of Oahu in Hawai'i were used in all experiments in this research. The final date of fruit harvest coincided within one week of commercial harvest.

CWI activity and fruit development: Fruit were picked every two weeks from 12 weeks before commercial harvest. Samples were handled and the activities of CWI were determined as described in Chapter 7.

CWI activity and translucency severity: Mature fruit were grouped into 4 categories, opaque (0%), low (10-30%), semi (40-60%), and high (>70%) translucency. Each group had three replicates with pooled sample of two fruit each. The activities of CWI were determined as described in Chapter 7.

Fruit distribution of CWI: Mature semi-translucent (40-60% severity) fruit were cut into three sections, top, middle and bottom. A randomized complete block design was used. Each section had four replicates. The activities of CWI were determined as described in Chapter 7.

Defoliation and CWI activity: Plants were defoliated 3 weeks before harvest. Total stem leaves were counted and 33.3, 66.7 or 100% of individual leaves removed from top younger ones downward. Flesh tissues from 4 fruit were diced and pooled together as one of three replicates. The activity of CWI of fruit flesh was determined as described in Chapter 7. This experiment was conducted from December 08, 1998 to December 28, 1998.

CWI activity in lodged fruit: Six lodged fruit were picked one week before harvest. Fruit were cut longitudinally into two portions, the adaxial (exposed-side; translucent) and the abaxial (shaded-side; non-translucent). The activity of CWI was determined as described in Chapter 7.

8.3 Results

The activity of CWI was high in the fruitlet 12 weeks before harvest and declined to a very low level 6 weeks before harvest (Figure 8.1). The CWI activity in the fruitlet increased again 4 weeks before harvest to a very high level at harvest. The CWI activity in the interfruitlet tissue was low throughout the development until 2 weeks before harvest.

The activity of CWI in the opaque pineapple fruit was significantly lower than that in the translucent fruit at harvest (Table 8.1) and a positive correlation existed between the activity of CWI and the severity of translucency. The activity of CWI was higher in the bottom section of pineapple fruit flesh than that in the top section (Table 8.2), and defoliation significantly reduced the CWI activity in the fruit flesh (Table 8.3). There appeared to be a cause-effect relationship between percentage of defoliation and the CWI activity (Table 8.4). The activity of CWI was lower in the adaxial side (exposed-side) of lodged pineapple fruit, that had translucent fruit flesh than on the abaxial side (shaded-side) with non-translucent flesh (Table 8.5).

8.4 Discussion

An increase in the activity of CWI, particularly in the fruitlet, in the last 4 weeks of pineapple fruit development (Figure 8.1), followed by the occurrence of translucency, implies a relationship between the CWI activity and translucency. This was also supported by the result that there was a positive correlation between the CWI activity and the severity of translucency (Table 8.1). In general, the basal flesh first showed translucency. The CWI activity in the bottom section of pineapple fruit flesh was higher than that in the top section

(Table 8.2), suggesting that the CWI activity was related to maturity, since the physiological age of the fruitlet in the bottom of fruit was ca. two weeks older than of the top fruitlet. The fruitlet generally showed translucency symptoms and accumulated sucrose earlier than the interfruitlet tissue (Figure 7.2). In addition, the CWI activity in the fruitlet also increased 2 weeks earlier during the last stage of fruit development (Figure 8.1). Leaf defoliation conducted at the latter stages of fruit development reduced TSS and translucency (Table 6.3 and 6.4), and CWI activity (Table 8.2) while crown removal had no effect (Table 6.1). Moreover, there was a cause-effect relationship between percentage of defoliation and translucency (Table 6.4) and CWI activity (Table 8.3). Taken together, a high activity of CWI in pineapple fruit flesh during the last stage of fruit development, that favored apoplastic phloem unloading, was a possible cause of pineapple fruit translucency.

Permeability to sugar across the plasma and tonoplast membranes in apple fruit flesh tissues increase with fruit maturation (Yamaki and Ino, 1992). Cells in immature apple fruit apparently enlarge through higher turgor pressure from import of sugars into vacuoles, and cease to enlarge in mature fruit as the amount of sugar unloading into the fruit is reduced due to the accumulation of sugar in the free space or cytoplasm (Yamaki and Ino, 1992). A breakdown of apoplast/symplast compartmentation, caused by declining cell membrane integrity, occurs around the time of onset of ripening in grape berries as the rate of phloem translocation to the fruit suddenly increases (Lang and Düring, 1991). Therefore, a high CWI activity in the apoplast would favor in maintaining an sucrose gradient between the phloem end and the apoplast in sink tissues, by cleaving sucrose into glucose and fructose. A high

osmotically active solute concentration in the apoplast will favor water movement into the intercellular free spaces that could lead to translucency occurrence.

The low CWI activity in the adaxial portion of lodged pineapple fruit (Table 8.3) was possibly due to thermal inactivation or heat damage of the enzyme, and suggested that translucency caused by heat stress was different from the maturity-related translucency. Pineapple fruit flesh became susceptible to high fruit temperature induced leakage (Figure 4.3) and translucency (Figure 4.2; Table 4.3 and 4.4) during the last stage of fruit development. In addition, increases in maturity-related leakage (Figure 4.2), sucrose accumulation (Figure 7.1) and CWI activity (Figure 7.4) also occur at the same stage. Therefore, the translucency symptom observed during the last stage of fruit development may be a combination of both effects. However, the maturity-related factors were the most common cause of translucency.

Table 8.1. Differences in the cell-wall invertase activity at various severities of pineapple translucent fruit flesh. The severity of translucency was subjectively evaluated on longitudinal sliced half fruit based on the percentage of affected area. Data are analyzed as a completely randomized design.

Translucency severity	Activity (μmol / h / g fresh weight)
Opaque (0%)	1.5 c
Low (10-30%)	4.3 bc
Semi (40-60%)	6.1 ab
High (>70%)	8.4 a
Analysis of variance F-test, probability	< 0.01
LSD 5%	2.8

Mean of 3 replicates.
Means followed by the same letter were not significantly different at the 5% level by the LSD test.

Table 8.2. Differences in the cell-wall invertase activity in various sections of pineapple fruit flesh. Mature semi-translucent (40-60% severity) fruit were cut transversely into three sections, top, middle and bottom. Data were analyzed as a randomized complete block design.

Fruit sections	Activity (μmol / h / g fresh weight)
Top	5.9 b
Middle	8.2 ab
Bottom	11.8 a
Analysis of variance F-test, probability	0.047
LSD 5%	4.4

Mean of 4 replicates.
Means followed by the same letter were not significantly different at the 5% level by the LSD test.

Table 8.3. Effects of defoliation at the last stage of fruit development on cell-wall invertase activity in pineapple fruit flesh at harvest. Removal of 33.3 to 100% of the stem leaves was conducted 3 weeks before harvest.

Treatments	Activity (μmol / h / g fresh weight)
100% defoliation	1.7 d
66.7% defoliation	2.9 c
33.3% defoliation	4.7 b
Control	10.5 a
Analysis of variance F-test, probability	< 0.01
LSD 5%	0.7

Mean of 3 replicates.
Means in the same column followed by the same letter were not significantly different at the 5% level by the LSD test.

Table 8.4. Linear regression analysis of the relationship between percentage of defoliation (D) 3 weeks before harvest and cell-wall invertase (CWI) activity in pineapple fruit flesh at harvest.

	R^2	Probability > F	Equation
CWI activity	0.866	< 0.01	CWI = 9.15 - 0.084D

Table 8.5. Differences in cell-wall invertase activity between the adaxial and abaxial sides of lodged pineapple fruit flesh. Fruit were picked one week before harvest. Data were analyzed as a randomized complete block design.

Fruit sections	Activity (μmol / h / g fresh weight)
Adaxial (exposed-side)	0.4
Abaxial (shaded-side)	2.1
Analysis of variance F-test, probability	< 0.01
LSD 5%	0.7

Mean of 6 replicates.

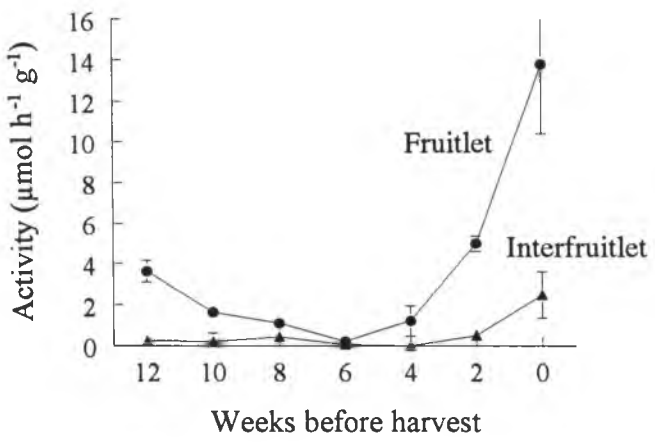


Figure 8.1. Differences in cell-wall invertase activity between fruitlet (■) and interfruitlet (□) tissues of pineapple fruit during development. Mean ± SD of three replicates.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

Paull and Reyes (1996) reported that the highest pineapple fruit translucency severity occurred in April and May, and lowest occurred in October, November and December. However, this was not found in this research possibly due to a variation in plant size and field location.

Pineapple fruit translucency began to occur 4 to 2 weeks before harvest (Figure 4.2) and followed an increase in membrane permeability of fruit flesh cells 6 to 4 weeks before harvest (Figure 4.3). The membrane permeability in translucent pineapple fruit flesh was higher than in the non-translucent flesh (Table 4.1). Covering fruit with clear-plastic during the last stage of pineapple fruit development increased the occurrence of fruit translucency (Table 4.3 and 4.4), possibly due to an increase in the membrane permeability of fruit flesh cells, since the susceptibility of fruit flesh to high temperature dramatically increased 6 to 4 weeks before harvest (Figure 4.2 and 4.3). As pineapple fruit matured and ripened, a loss in cell membrane integrity could lead to an increase in membrane permeability, that, in turn, would lead to an increase in apoplastic solute levels, such as in ripening grape berries (Lang and During, 1986).

Plants cells have the ability to increase thermotolerance when grown for a long period at elevated temperature or when subjected to a transient heat stress (Santarius and Weis, 1988). The temperatures to which apples have been previously subjected play a significant role in determining the degree of apple heat resistance during maturation and that ability to

endure high temperature can be greatly increased by gradual hardening to heat (Brooks et al., 1926). During early pineapple fruit development, high daytime fruit temperature could possibly increase the fruit heat tolerance, that may reduce the occurrence of fruit translucency during the last stage of fruit development. The membrane permeability of young pineapple fruit flesh can be reduced by covering fruit with clear-plastic in the field (Table 4.5) or a postharvest heat treatment (Figure 4.3) implying an increase in heat tolerance. As a result, the reason that there was a significantly negative correlation between air temperature 3 to 2 months before harvest and fruit translucency at harvest during the year (Paull and Reyes, 1996) was possibly because high temperature 3 to 2 months before harvest increased the heat tolerance of the fruit flesh cell membranes, and that may reduce the occurrence of fruit translucency during the last stage of fruit development.

Calcium plays an important role in the maintenance of the membrane integrity, middle lamella and cell wall rigidity (Leshem, 1992; Brett and Waldron, 1996). Calcium concentration in pineapple fruit flesh declined with development (Figure 5.1) that was consistent with the results found in apple (Wiersum,1966: Ferguson and Watkins, 1989) and tomato (Ho et al., 1987). The ability of divalent cation binding of cell wall in pineapple fruit flesh also declined with development (Figure 5.2). Pineapple fruit translucency has been suggested to be related to an increase in cell wall hydrolases (Soler, 1993) and membrane permeability (Soler, 1994). High calcium concentration may decrease the secretion or activities of cell wall hydrolases (Huber, 1983) and membrane permeability. Preharvest calcium sprays and postharvest pressurization of calcium have been used to increase fruit firmness and reduce decay or calcium-related disorders (Leshem, 1992). In pineapple,

spraying calcium during fruit development increased the calcium concentration in fruit flesh (Table 5.3) and decreased the occurrence of translucency (Table 5.1 and 5.2).

The correlation between crown weight and translucency severity during the year is significantly negative (Paull and Reyes, 1996) due possibly to the crown and fruit being competitors for photoassimilates, nutrients, water, etc. Small fruits generally have large crowns and vice versa. Forcing at high night temperature reduces eye numbers and fruit weight, but increases crown weight, with the total weight of fruit and crown being unaffected (Min and Bartholomew, 1997). In addition, applying chlorflurenol to reduce crown size did not affect the fruit size (Keetch and Dalldorf, 1980; Dalldorf, 1981). This suggested that pineapple fruit was a stronger sink than the crown during fruit growth and development, and consistent with other observations that, in general, fruit are stronger sinks than the vegetative organs for assimilates (Ho, 1988). Removing the crown either at an early or late stage of pineapple fruit development did not cause any significant effect on fruit weight and translucency (Table 6.1), suggesting that the crown does not play a significant role in pineapple fruit development and translucency occurrence, and there was no cause-effect relationship between crown size and fruit translucency though there was a significant correlation between crown weight and translucency severity during the year.

The sink tissue apoplast can serve as a storage region for sugars; 13% of the total sugars in strawberry fruit (John and Yamaki, 1994) and 20% of the sucrose in mature sugarcane tissue (Welbaum and Meinzer, 1990) are held in the apoplast. An increase in the apoplastic solute level in the fruit flesh could promote removal of water from the phloem into the apoplast due to an increase in the osmotic pressure contributed by the imported solutes

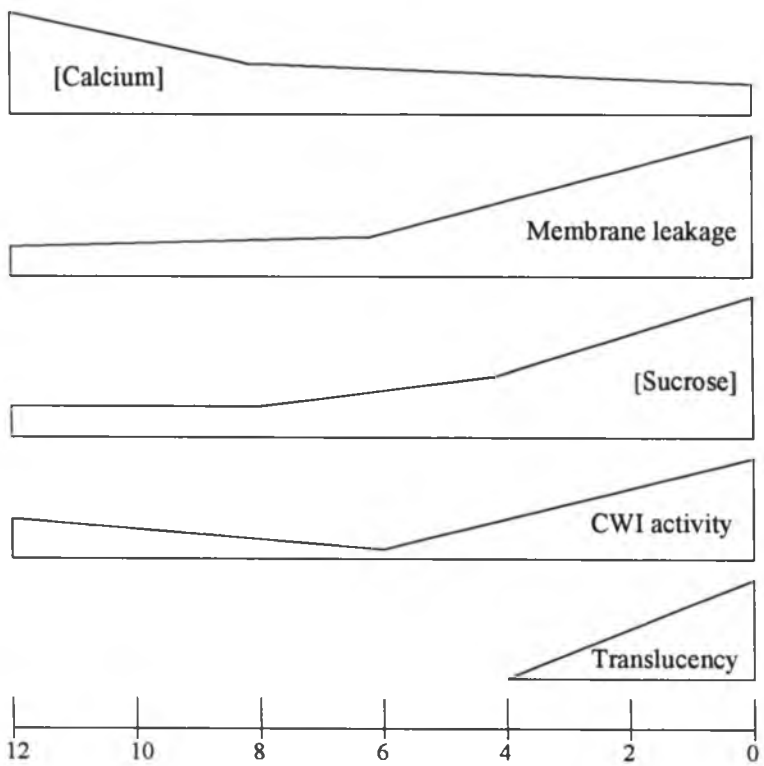
(Lang and Thorpe, 1986). The higher apoplastic osmotic pressure and water uptake could favor the occurrence of pineapple fruit translucency.

Defoliation conducted 3 or 4 weeks before harvest decreased the pineapple fruit flesh TSS and translucency (Table 6.2 and 6.3). There was a linear cause-effect relationship between percentage of defoliation and TSS and translucency (Table 6.4), suggesting that photoassimilate unloading in fruit flesh during the last stage of fruit development played an essential role in the occurrence of pineapple fruit translucency.

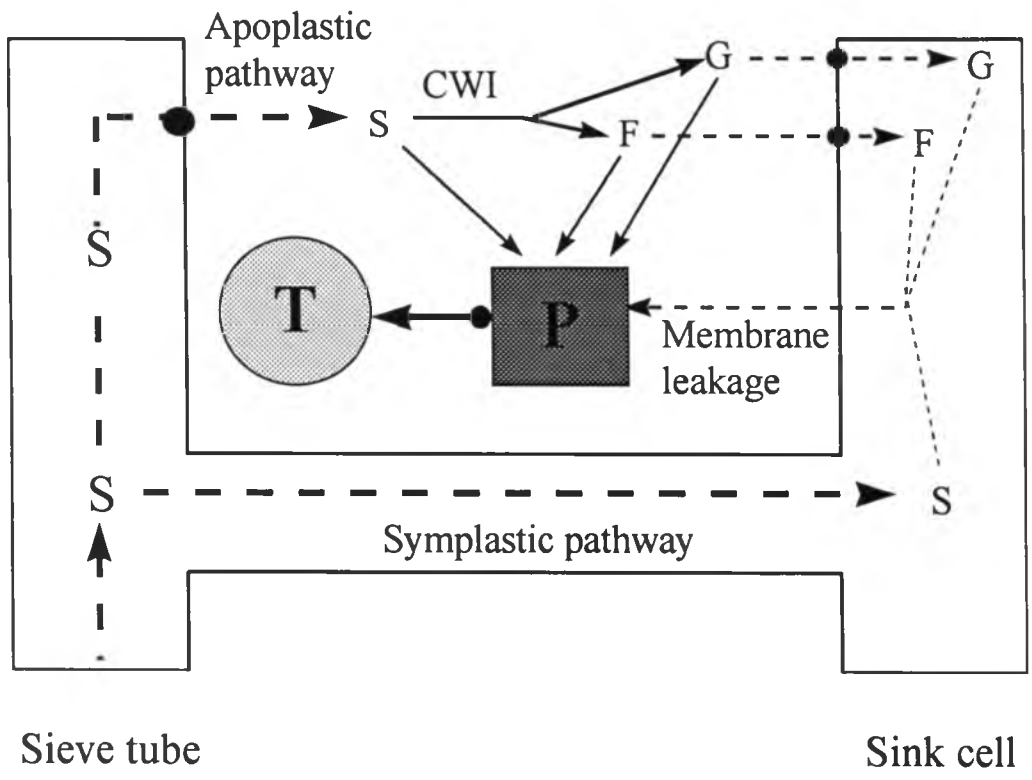
Sucrose began to rapidly accumulate in pineapple fruit flesh 6 weeks before harvest (Figure 7.1), concomitant with decreases in activities of acid, neutral and cell-wall invertase, and sucrose synthase (Figure 7.3 and 7.4). Cell-wall invertase (CWI) activity in pineapple fruit flesh increased again 4 weeks before harvest (Figure 7.4) and was followed by the first symptom of translucency (Figure 4.2). There was a positive correlation between the CWI activity and translucency severity (Table 8.1). The results suggested that the high CWI activity, favored apoplastic phloem unloading was one of the causes inducing the occurrence of pineapple fruit translucency. Fruitlet and the basal flesh generally was the first to show translucency, and had higher sugar content and CWI activity compared to the interfruitlet and top flesh, respectively, suggesting that translucency was related to maturity. This maturity-related translucency was different from the heat stress, caused by high solar radiation, that induced translucency commonly observed in lodged fruit.

Pineapple fruit translucency occurring at the latter stages of fruit development was due to a combination of effects related to high fruit temperature and fruit maturity. Associated factors affecting the severity of translucency include a decrease in calcium concentration of

fruit flesh and of divalent cation fruit flesh cell wall binding ability that lead to a loss of cell membrane integrity and cell wall fragility. These changes were followed by an increase in membrane permeability and an enhanced susceptibility of fruit flesh to high temperature. Increased sucrose accumulation and the activity of CWI favoring apoplastic phloem unloading, caused an increase in the solute concentration and liquid volume in the apoplast, that in turn lead to translucency. The results can be summarized as the following figure.



Taken together the above results, the following model has been proposed that can be tested further.



S: sucrose; F: fructose; G: glucose; CWI: cell-wall invertase; T: translucency; P: osmotic pressure.

There are four control steps in this model:

1. The sucrose unloading from the sieve tube to the apoplast.

2. The activity of cell-wall invertase that cleaves sucrose into glucose and fructose.

3. The uptake of glucose and fructose by the sink cells from the apoplast.

4. The membrane permeability of sink cells.

Any factor favoring an increase in osmotic pressure in the apoplast could lead to the occurrence of translucency.

APPENDIX

WEATHER DATA DURING THE PERIOD OF THIS RESEARCH

Weather data obtained from the UH Poamoho station, 1~2 miles from the Dole's field used in this research.

Year: 1996

Month	Rain fall (inches)	Temperature (°C)	
		High	Low
January	6.56	26.5	18.4
February	4.92	23.4	16.9
March	5.99	24.4	16.8
April	2.22	27.9	19.5
May	1.44	27.0	19.8
June	4.85	28.7	21.3
July	---	---	---
August	2.44	30.8	21.7
September	6.10	29.4	21.9
October	1.39	30.2	21.4
November	26.71	26.4	20.7
December	8.38	24.5	18.4

Year: 1997

Month	Rain fall (inches)	Temperature (°C)	
		High	Low
January	14.36	23.6	18.2
February	1.37	24.9	18.9
March	6.45	24.5	19.1
April	7.17	25.6	19.3
May	2.61	24.9	19.0
June	0.98	27.0	21.6
July	1.05	27.2	21.4
August	0.79	29.0	21.5
September	2.59	29.4	21.8
October	1.15	29.0	21.4
November	4.08	25.9	19.3
December	1.95	24.4	18.5

Year: 1998

Month	Rain fall (inches)	Temperature (°C)	
		High	Low
January	3.09	24.8	17.2
February	1.31	25.7	17.0
March	0.64	26.0	17.9
April	3.08	25.0	18.4
May	1.06	25.9	19.1
June	0.94	27.1	20.2
July	0.42	28.2	20.5
August	0.59	29.1	21.3
September	0.32	29.3	20.9
October	0.56	28.8	21.0
November	2.82	26.7	20.7
December	2.42	25.7	18.9

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